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FEEDBACK BY THE SOIL MICROBIAL COMMUNITY ON THE DISTRIBUTION OF TWO WEEDS

BY

YI LOU

THESIS

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Adviser:

Assistant Professor Anthony Yannarell

ABSTRACT

Soil microbial communities may influence the composition and species abundance of plant communities through the phenomenon of "soil feedback," which is the tendency of a plant species to cause physical, chemical and/or biological changes in the soil that are beneficial or detrimental to its own fitness or that of its competitors. In this study, I investigated the microbial communities associated with soil feedback to two agricultural weeds, common sunflower (*Helianthus annuus*) and giant ragweed (*Ambrosia trifida*). These two plants generally follow opposite gradients of abundance from west to east in the northern United States, and I hypothesized that soil feedback can partially explain this pattern. In each of six states, sunflower and ragweed were grown separately in local soil for two 10-weeks "soil training" phases in order to allow each plant species to modify the microbial communities. Next, sunflower and ragweed were grown separately in the soil trained by the same species and also in the soil trained by the other species, and a soil feedback score was calculated by comparing the biomass of the plants in these two treatments. Bacterial and fungal communities at the end of the experiment were assessed by automated ribosomal intergenic spacer analysis (ARISA). I evaluated the relative effects of three forces shaping microbial communities: 1) the source community, which was the initial microbial community present in the local soil of each state; 2) the influence of the training plant species (ragweed or sunflower) in the initial phase of the experiment; and 3) the influence of the plant species (ragweed and sunflower) in the final phase of the experiment. I also used multivariate modeling to identify the key microbial taxa that contributed to the feedback score calculated from the plant biomass. I found that the source community had the strongest effect on microbial community composition. This suggests that the plant - soil feedback largely depends on the starting microbial communities that the plant encounters. However, within each state, I could detect a significant influence of the plants on microbial community composition over the course

of the experiment. Of the microbial taxa that responded to plant influence, only a few were identified by multivariate modeling as being significantly related to plant growth (i.e. soil feedback). Only 10% of the taxa in the total microbial community were needed to classify soil feedback as either net positive or net negative, and these same key taxa could predict the observed feedback scores for sunflower and ragweed ($R^2 = 0.80$). Furthermore, some key taxa may be involved in interactions between ragweed and sunflower. Sunflower growth increased the abundance of bacteria that significantly affected ragweed growth. However, ragweed growth generally increased the abundance of bacteria that only affected ragweed. For each plant species, beneficial fungal taxa were more abundant in the soils of states where the plant received positive soil feedback, and harmful fungal taxa were more abundant in the soils of states where the plant received negative feedback. Another difference between bacteria and fungi is their apparent response to plant selection pressure. Bacterial community variability was reduced in treatments with large positive or large negative feedback scores (P=0.02), but the same was not true for fungi. Overall, my results show that ragweed and sunflower's capacity to shape soil microbial communities is strongly dependent on the source community, which varies over the geographic range of these weeds. A minor fraction of the soil microbial community appears to be involved in soil feedback. Beneficial fungi for sunflower tend to be found in places where sunflower abundance is highest, while harmful fungi are found in places where sunflower abundance is lowest. The same is true for ragweed. However, soil bacteria appear to respond more strongly to plant selection and may be involved in interactions between sunflower and ragweed. A better understanding of the geographic variability of microbial communities, the key microbial taxa that respond to plant selection, and their feedback to plant growth, may lead to more effective management strategies for agricultural weeds.

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Introduction

Understanding weed problems is vital to agricultural management. Weeds threaten crop yields and cause approximately 10% of crop loss in the USA (Chee-Sanford, 2008). Weed control methods, such as suppressing weed seed production, selection of a crop with the capability of suppressing weeds, and herbicide applications, applied alone or in combination, have been developed and used widely; however, weeds still persist in agricultural systems (Swanton & Weise, 1991). One serious problem obstructing the efficiency and capability of weed management is the limited knowledge of the distribution of weed populations across the landscape (Cardina et al., 1997; Garibay et al., 2001). Weed scientists have recognized that annual weed populations within fields are usually highly variable (Freckleton & Watkinson, 2002). Thus, increasing attention toward reducing the dependency on herbicides has heightened interests in weed management strategies that combine the knowledge of the suite of factors causing weed distribution variation in different sites (Buhler. et al., 1997; Myers et al., 2005). Successful weed management needs to address one important question: why does a given weed species succeed under one soil condition but not in others (Cardina et al., 1997)?

1.1 Agricultural weeds and the microbial soil community

Many factors closely related to the soil microbial community are changed by agricultural practices. Furrow irrigation (Wang et al., 2008), addition of crop residues to the soil (Collins et al., 1992), and using herbicides for weed control (Garcia-Orenes et al., 2010), can dramatically change soil structure, soil biochemical cycling and plant communities. As a result of these activities, soil microbial communities could be

impacted. For example, deep plowing or drainage of carbon-rich soil is known to stimulate rates of decomposition and respiration, because it enables microorganisms to access both oxygen and deeply buried organic carbon (Singh et al., 2010). In addition to the impacts from physical practices, soil chemical management techniques, like fertility applications, are also important factors that could considerably change agricultural soil microbial communities. Low-nitrogen-input agriculture may promote the growth of oligotrophic communities, because oligotrophs are considered slow-growing and dominant in nutrient-limited ecosystems (Monson et al., 2006). These agricultural practices alter soil microbial communities. When the altered soil microbial communities come into contact with plants, the microbial communities may provide a direct and indirect influence over the growth of the crop (Harrier & Watson, 2003; Machiavelli et al., 2008) and the weed communities (Jordan & Huerd, 2008) in agroecosystems.

A recent review (Caesar, 2005) has discussed a number of links between microbial activities and weed communities in agroecological systems. For example, it is well known that crop rotation can reduce species-specific soil pathogen accumulation in the field (An et al., 1993; Schmitt, 1991). The altered soil pathogen community may also influence weed-pathogen interactions in surrounding fields (Tian & Babadoost, 2004). Microbes targeting specific weeds take advantage of the microbe-weed interactions. People have used soils containing “mycoherbicides” (fungi used as herbicides), cultured fungi or bacteria as “bioherbicides” to depress target weed growth (Vatovec et al., 2005). Additional, sustainable management practices suggest that the deleterious rhizosphere bacteria (DRB) communities should be incorporated into crop management for the biological control of weeds. The DRB inhibit specific weed species and then allow crops to out-compete the suppressed weeds (Kremer & Kennedy, 1996).

Microorganisms could also impact target weed community through influencing the weed seed bank. Germination and initial seedling growth might be inhibited by diverse

seedborne bacteria (Kremer 1987) and fungi (MacDonald & Kotanen, 2010). The mechanisms of microorganisms impacting weed seed banks include microorganisms that directly penetrate the seed (Kremer et al., 1990), damage the seed coat (Mortensen & Hsiao, 1987) and release metabolites that are toxic to weed seeds (Halloin et al., 1991). These studies suggest significant effects of microbial communities in driving annual weed community composition and variation.

1.2 Ragweed and sunflower distribution and plant soil feedback

1.2.1 The variation of ragweed and sunflower distribution

Prior to my thesis work, my collaborator in the NC1026 - North Central Regional Committee on Weed Biology project (Buhler, 2005) conducted demographic study of two model weeds that are common and dominant in US agriculture ecosystems. These two weeds, common sunflower *(Helianthus annuus)* and giant ragweed *(Ambrosia trifida*), were selected for study for following reasons. First, these two weeds are among the most competitive weeds in their ranges, and they have shown resistances to herbicides (Buhler, 2005). Also they have large seed, which permit accurate seedbank measurements (Buhler, 2005). Interesting, the primary results of NC1026 demographic survey (Buhler, unpublished data) demonstrated that common sunflower and giant ragweed generally followed opposite gradients from west to east in both abundance and weediness; the abundance and weediness of sunflower decreases as one moves eastward, and ragweed is the reverse. A series of "home vs. away" feedback experiments suggested an opposite direction of feedback scores between sunflower and ragweed in Illinois, Kansas, South Dakota, Oregon, Montana and Michigan (Buhler, 2005)

Why do ragweed and sunflower populations show opposing patterns of distribution in the agricultural ecosystem of the north central region? The answer to this question likely

involves a myriad of associated environmental factors that contribute to a plant's ability to spread, dominate a community and displace their competitor's niche while altering the quality of the habitat. Much of the research investigating the mechanisms influencing spatial variation of weed populations has focused on herbivore effects (McEvoy, 2002), seed bank emergence and persistence (Buhler et al., 1997; Swanton & Weise, 1991) or have considered the nutrient content and physical properties of soil (Buhler. et al., 1997; Burton et al., 2005; Swanton & Weise, 1991). Soil chemical and physical properties have had limited ability to explain weed species variability across landscapes (Dieleman, Mortensen, Buhler, Cambardella, et al., 2000; Dieleman, Mortensen, Buhler, & Ferguson, 2000).

In response to the perceived limitations of explaining weed species' variation through plant above-ground interactions and soil properties, weed ecologists have increasingly looked for mechanisms that are specific to belowground microorganisms determining spatial variation of weed community structure and diversity. For instance, microbial pathogens were introduced to control weed species (Berestetskiy, 2004; Caesar, 2005). Weed seeds often selectively associate with microorganisms (Chee-Sanford, 2008; Dalling et al., 2011). The different infections of microbial species on the seed are thought to relate to the protection or germination of the seed, which would influence the weed community (Chee-Sanford et al., 2006; Dalling et al., 2011). Better performance of Arbuscular mycorrhizal fungi -host weed species than non-host species indicated weeds can be strongly affected by the fungal component of soil microbiota (Jordan & Huerd, 2008). Therefore, to understand weed community variation more thoroughly, a microbial perspective needs to be integrated into the knowledge framework.

1.2.2 Soil microbial community feedback

The term “plant-soil feedback” was developed to integrate a microbial perspective into the traditional framework of plant ecology (Bever, 2003; Bever, Westover, & Antonovics, 1997). The feedback process involves two steps. First, a plant or plant population can directly and indirectly change its associated community of soil microorganisms; second, the altered microbial communities will in turn generate effects on plant growth and fitness. The range of soil microorganisms' direct effects on plant species ranges from positive (e.g. Nitrogen fixing bacteria, arbuscular mycorrhizal fungi) to negative (e.g. pathogenic bacteria and fungi). The feedback framework, in both theory and practice, has been proven to be useful in assessing the interactions between soil microbial communities and plant communities in a Canadian grassland (Klironomos, 2002), US serpentine and prairie soil (Casper et al., 2008), and subtropical areas (Te Beest et al., 2009). The soil community feedback provides an important mechanism for the modification of plant communities (distinct from soil resource or niche partition theory) through the changes in the composition and abundance of mutualistic and pathogenic microorganisms that have beneficial or deleterious effects on plant species (Bever et al., 2010; Reynolds & Haubensak, 2009). The direction and magnitude of feedback score, the effects on the growth of plants mediated by soil biota, could positively correlate with plants' relative abundance in communities (Klironomos, 2002; Mangan et al., 2010; Reinhart et al., 2003).

1.3 Ragweed, sunflower and soil microbial communities

Both common sunflower *(Helianthus annuus)* and giant ragweed *(Ambrosia trifida)* are widely found in North American crop fields, and are considered to be important agricultural weeds in their respective geographic ranges (Anderson, 2007; Baysinger &

Sims, 1992; Forcella et al., 1997). Sunflower and giant ragweed differ in their responses to various soil abiotic factors (Anderson et al., 1998; Cummings et al., 2002; Myers et al., 2005), but both have shown great potential for altering the soil microbial communities directly and indirectly (Fu & Cheng, 2004; Fumanal et al., 2006; MacKay & Kotanen, 2008). Microbial communities can generate feedback to modify plant communities through the dynamics of specific soil microbial taxa, resulting in enhanced weed performance (Fumanal et al., 2006) or reduced weed survival (Chee-Sanford, 2008). Accordingly, altered soil microbial conditions could influence weed distribution and abundance (Anderson, 2007; Caesar, 2005). The study of soil microbial communities associated with weeds will help explore the underlying dynamics and distribution of weeds through a soil feedback perspective.

1.3.1 Ragweed

Giant ragweed (*Ambrosia trifida*) is an aggressive annual weed and is capable of high seed production and domination of plant communities by suppressing associated species. It can cause significant reductions in crop yield (Baysinger & Sims, 1992; Harrison et al., 2001). In the northwest corn belt, giant ragweed is among the most competitive annual weeds of soybean and corn (Gibson et al., 2006). The factors that contribute to giant ragweed's success include its temporal emergence pattern (Myers et al., 2005), variable emergence depth in soil (Harrison et al., 2007), rapid and aggressive growth, and high degree of morphological and reproductive plasticity in response to the encroachment by neighboring plants (Harrison et al., 2007; Harrison et al., 2001). Giant ragweed is not only considered a severe weed in North America, but it is also a dangerous invasive species on the Eurasian continent (MacDonald & Kotanen, 2010; MacKay & Kotanen, 2008; Pajevic et al., 2010). Its invasive ability is related to its higher photosynthetic rates

than noninvasive species when growing under unfavorable conditions (Pajevic et al., 2010).

In addition to the evidence from soil properties and resource competition, soil microbes are important for the variation of ragweed populations in different areas. Ragweed roots can be highly colonized with arbuscular mycorrhizal fungi in both native and invasive areas. Fumanal found this AMF association could facilitate common ragweed invasion in various habitats in France by increasing plant biomass, pollen and seed numbers (Fumanal et al., 2006). In nature, ragweed populations can even reside with surprisingly low (1%) root AMF colonization levels (Fumanal et al., 2006). The wide adaption to mycorrhizal colonization levels gives ragweed an advantage for spreading within a new area. Additional, fungal species can decay weed seeds to obtain nutrients in the soil (Kremer, 1993). Fewer fungi have been found on ragweed seeds than on those of velvetleaf (Chee-Sanford, 2008). This may explain why extensive decay of ragweed seed was not observed in the field (Davis, 2007). Thus, the germination of ragweed appears to be related to the microbial community associated with the seed.

1.3.2 Sunflower

The other annual weed considered in this study is the common sunflower (*Helianthus annuus*), a native North American weed species. It is prominent in the corn belt, and can reduce corn and soybean yields considerably (Forcella et al., 1997). In Kansas, soybean yield reduction ranged from 17-19% with low sunflower density, to 95-97% with high density (Geier et al., 1996). Sunflower shows a high competitive advantage for soil moisture, nutrients (Burton et al., 2005) and light (Geier et al., 1996) over agricultural crops.

Sunflower has been shown to influence soil respiration (Fu & Cheng, 2004) and change rhizosphere microbial community composition (Kamal & Bano, 2008; Staman et al., 2001), which may alter soil microbial activities in ways that will feed back to sunflower growth. Soil microbial respiration is related to the defoliation of sunflower (Fu & Cheng, 2004). Sunflower rhizosphere respiration and associated soil microbial respiration were significantly enhanced by high level defoliation as compared to unclipped plants. The increased rhizosphere and soil microbial respiration were strongly related to stimulation of soil organic matter decomposition (Fu & Cheng, 2004). The allelopathic effects of sunflower have greatly attracted researchers' attention since the effects can strongly inhibit the growth and development of other crops (Azania et al., 2003; Ciarka et al., 2009). It is likely that this high allelopathic ability of sunflower could change soil microbial communities as well. Compared to the control, potential allelochemicals greatly decreased the number of colonies of *Azospirillum* (a phosphate-solubilizing bacteria isolated from sunflower roots) and *Rhizobium* (a bacteria isolated from sunflower rhizosphere) (Kamal & Bano, 2008). Field soil mixed with sunflower leaves could stimulate the community of phenolic acid-utilizing rhizosphere bacteria. This demonstrated that certain bacterial species in the rhizosphere respond to a plant allelopathic chemical transferred from plant residuals to the rhizosphere (Staman et al., 2001). However, at present, there are few studies of the direct relationships between allelopathic effects of sunflower and general soil microbial communities based on uncultured molecular methods.

1.4 Feedback and composition of plant communities

Soil community feedback was suggested to provide an important mechanism of modification of plant communities (Bever et al., 2010; Klironomos, 2002). The feedback caused by the changes of soil microbial community has been found to positively correlate

with the plants' relative abundance (Klironomos, 2002; Mangan et al., 2010; Reinhart et al., 2003). The theory of soil community feedback suggests that positive feedback tends to destabilize plant coexistence, because it strengthens the beneficial relationship between the dominant plant species and the soil community (Bever, 2003). This will lead to an increased abundance of plant species. In contrast, the negative feedback can decrease or restrict the abundance of a dominant species. Numerous studies have shown that tree seedlings received negative feedback from conspecific adults, since seedling performance is significantly reduced when grown in the presence of adult trees (Mangan et al., 2010; Packer & Clay, 2000; Reinhart et al., 2003). Therefore, negative soil feedback can promote the possibility of species' coexistence and diversity by preventing the same species from occupying a community (Bever, 2003; Reinhart & Callaway, 2006).

1.5 Factors influencing soil microbial community feedback

1.5.1 Source microbial community

Soil microbial communities exhibit distinguishing biogeographic patterns at the continental scale (Fierer & Jackson, 2006). The different microbial species or communities often form specific relationships with host plant species (Bever, 2002; Van der Putten et al., 1993). For example, plant species show host-specific associations with different arbuscular mycorrhizal fungi (AMF). Distinct AMF communities were generated by associating with different hosts. These altered AMF communities could then differentially feed back to host plant species' growth (Bever, 2002; Bever et al., 1996). The same pattern was also reported from other soil microorganisms, such as pathogens (Van der Putten & Peters, 1997). Additionally, the impacts that different plant species received from one soil organism can vary greatly. For instance, eleven plant species responded differentially to AMF association (Adjoud et al., 1996). There is a high

variance in the range of plant growth responses to local and exotic AMF species (Klironomos, 2003). As a result, plant–soil community feedback highly depends on the initial microbial community that plant species can interact with.

The source microbial community composition plays an important role in plant invasion. Plant species invade by encountering beneficial or less deleterious soil communities in the habitat. Feedback may become positive for a plant species because it was introduced into regions with fewer soil-borne enemies than are encountered in its native habitat; this is a critical aspect of the enemy release hypothesis (Diez et al., 2010; Mitchell & Power, 2003). Other invasive species encounter novel and strong soil mutualists that enhance their invasive success. This process is not clearly documented, but it is possible that a nonnative plant can build a new mutualistic relationship, so that the plant could spread within the new habitat (Reinhart & Callaway, 2006; Reinhart et al., 2003). Moreover, the different species' composition of plant enemies between native and nonnative source communities may give a plant an opportunity to invade. Plant species released from native pathogens are harmful invaders in both agricultural and natural ecosystems (Mitchell & Power, 2003). The starting microbial communities that plants encountered significantly influence the feedback outcomes.

1.5.2 Key microbial drivers in feedback

Not every microbial species in the whole community is identified as an effective agent of feedback. Actually, a few microbial taxa may drive or dominate the key processes in feedback interactions. Key microbial drivers, such as pathogens, cause significantly negative feedback responses in plants (Klironomos, 2002). Mutualistic mycorrhizal fungi or beneficial bacteria help plant species spread in new habitats (Fumanal et al., 2006; Van der Putten, Kowalchuk, et al., 2007). Although considerations

on any singular microbial driver of soil feedback on plants would likely lead to erroneous assumptions, focusing on the "keystone" species in the plant-soil community feedback will help us dissect some components of the whole community and partition biological interactions into different functional groups (Ehrenfeld et al., 2005; Reinhart & Callaway, 2006).

1.5.2.1 Arbuscular mycorrhizal fungi (AMF)

Arbuscular mycorrhizal fungi have been traditionally known to facilitate soil water availability, increase uptake and exchange of nutrients (especially phosphorus), and provide pathogen resistance for their associated plants (Harrier & Watson, 2003; Van der Putten et al., 2001). This can influence the plant community. Soil AMF are important mediators of interactions between pairs of competing plant species (Bever et al., 1996; Pringle et al., 2009). An alien plant species in a new area can benefit from the widespread geographical distribution of AMF and their low host plant specificity (Fumanal et al., 2006; Mitchell et al., 2006). Invasive plants can also gain advantages by degrading native AMF associations (Stinson et al., 2006; Vogelsang & Bever, 2009). Some invasive species are not mycorrhizal hosts or are less mycorrhizally- dependent (Vogelsang & Bever, 2009). Thus they devote fewer resources toward maintaining mycorrhizal association than natives, and they may devote more resources toward growth and competition (Vogelsang & Bever, 2009; Zhang et al., 2010). In all, plants that are less dependent on mycorrhizal associations may gain a growth advantage over mycorrhizal plants by assigning less energy to maintain the mutualism.

1.5.2.2 Beneficial bacteria

Beneficial soil bacteria enhance host plant fitness and growth either by providing various forms of resources (e.g. nitrogen-fixing bacteria provide nutrients) (Reynolds et al., 2003; van der Heijden et al., 2008), or by stimulating phytohormone synthesis (Costacurta & Vanderleyden, 1995). In agricultural systems, the performance of four economic plants was significantly increased by associating with plant growth-promoting bacteria (PGPB) compared to plants that were uninoculated (Furnkranz et al., 2009). The PGPB showed potential for nitrogen fixing and phytohormone synthesis (Furnkranz et al., 2009). Bacterial communities can regulate the nitrogen transformation processes (Konopka, 2009) that play an important role in resource utilization among plant species (Ashton et al., 2008; Vitousek & Walker, 1989). In addition, plants can alter the availability of different chemical forms of nitrogen through microbially-mediated feedback (Reynolds & Haubensak, 2009). Coexistence of two alpine grassland plants species enhanced ammonium uptake over that of a single plant species. This observation could be explained by the increased rhizosphere extracellular enzyme activities. This result suggested that microbial community, in response to specific plants, facilitated plant coexistence by enhancing nitrogen uptake (Ashton et al., 2008).

Beneficial bacteria also promote plant growth by suppression of plant pathogens through synthesis of antimicrobial compounds or induction of resistance in the plant (van Loon, 2007). The rhizobacterium *Bacillus* could ameliorate the impacts of fungal pathogen on two plant species, *Panicum sphaero-carpon* and *Anthoxanthum odoratum*. One plant species grew relatively better with *Bacillus* that was isolated from the other plant species than from itself. This indicates that certain associations with benefical bacteria may enhance the performance of competitor plant species, resulting in a net negative outcome for the host plant, a form of negative feedback (Westover & Bever, 2001).

1.5.2.3 Pathogens

The specificity of host-pathogen interactions can lead to disparities in negative feedback. Pathogens involved in negative feedback play important roles in plant range expansion and invasion. Invaded plants escape their enemies in home sites and accumulate fewer local pathogens in new areas than the native species. This is a critical aspect of enemy release hypothesis for plant invasion (Diez et al., 2010; Mitchell & Power, 2003).

Some exotic plants suffer less negative feedback from soil biota than native species do. The exotic grass *Cenchrus biflorus* has neutral to positive feedback from soil-borne pathogens; the native grasses *Eragrostis lehmanniana* and *Aristida meridionalis* are subject to neutral or negative feedback effects (Van der Putten, Kowalchuk, et al., 2007). In contrast, a study found the effects of fungal pathogens on buried seeds were not significantly different between natives and exotics (Blaney & Kotanen, 2002). Similar conflicting results of plant-pathogen interactions were also observed in a two-year study (Agrawal et al., 2005). Data obtained in 2003 (Agrawal et al., 2005) suggested that exotic plants suffered less from fungal and viral pathogens than native species did; however, the pathogen effects on the plants were the opposite in 2002. Possible explanations for this inconsistent result are the variability of pathogens across time and the genetic changes in the plant community. Negative feedback caused by pathogens may contribute to the succession of plant species: late succession plant species were more tolerant to the soil-borne diseases than their preceding species (Van der Putten et al., 1993).

The different associations of plant species with pathogens could represent evolutionary pressure for plant communities. (Van der Putten et al., 1993; Van der Putten et al., 2001). Plant species responded differently to the growth-depressing microorganisms during succession. *Ammophila. arenaria* (Marram grass) and *Hippophae rhamnuides* (sea

buckthorn) grew better in soils conditioned by early successional plants than in soils conditioned by later successional plants. This is because soil borne pathogens accumulated in the soil during succession. This indicated soil pathogens could facilitate the succession of plants by disfavorably affecting host plant species (Van der Putten et al., 1993).

1.5.3 Microbial community variability

Previous work has shown that plant species are likely to exert strong selective pressures on the soil microbial community through rhizodeposition, root senescence, and litter deposition (Grayston et al., 2001). The selective pressure exerted on the soil microbial community may specifically select certain microbial species, which will reduce microbial community variability (S.J Graystona, 2004). The accumulation of specific microbial groups (pathogens or benefactors) can also generate feedback effects (either positive or negative) to host plants (Bever, 2003; Vogelsang & Bever, 2009). Therefore, it could be hypothesized that the microbial community variability should be negatively correlated with the magnitude of feedback because of accumulations of potential pathogens or benefactors.

Small variability of the microbial community may be a result of the accumulation of specific microbial taxa in the community. Inhibitory and beneficial effects of soil microbes on plants depend on the net effects of accumulated pathogenic and beneficial soil organisms. This accumulation of specific microbial taxa may reduce community variability. For example, the positive feedback plant *Chromolaena odorata* accumulates soil pathogens that inhibit other plants (Mangla et al., 2008). Plants grown in soil pre-cultivated by the same species, often show reduced performance, which is commonly attributed to the accumulation of inhibitory soil biota (Diez et al., 2010; Packer & Clay,

2000). Similarly, soil microbes can enhance the success of plants in new areas, such as *Bidens pilosa* (Cui & He, 2009) and *Myrica faya* (Vitousek & Walker, 1989), by enhancing or accumulating beneficial microbes.

1.6 Explanation of objectives

Given the rapid development of microbial feedback research and the close connection to weed species performance, weed ecologists have recognized the importance of mutual interactions between soil microbial communities and plant communities. They have begun to adapt and revise classical weed biocontrol strategies by taking microbial feedback into consideration (Caesar, 2005). However, most of the previous research on soil microbial communities in weed management focuses on the effects of AMF on weed density and biomass (Jordan & Huerd, 2008), or on specific pathogens infecting plants in the farm field (Jordan et al., 2000; Vatovec et al., 2005). In fact, the number of microbial species in soil is much greater than people have cultured and described (Torsvik & Ovreas, 2002). Thus, relatively little is known about the whole microbial community as a driving force of weed community variation in agricultural systems, and whether this force is sufficient and consistent to influence weed communities under quite different soil conditions (Jordan & Huerd, 2008; Quimby et al., 2002). These questions require comprehensive analysis for microbial diversity and composition that are related to the weed community. In addition, current trends in management of field-crop agroecosystems are promoting practices that tend to stabilize the soil environment, which will likely encourage the importance of weed–soil microbe interactions and soil microbial community feedback to weed growth (Caesar, 2005; Quimby et al., 2002).

My research represents a new inquiry into the question of whether soil microbial communities can explain the positive or negative feedback to ragweed and sunflower. I

took advantage of a feedback experiment conducted by my collaborators in the NC1026 Weed Biology Working Group and used microbial molecular methods to study bacterial and fungal communities from each soil pot.

The compiled dataset for this study was large, encompassing microbial ARISA data and experimental information from six states. The treatment variables included initial differences in microbial communities from different states, community differences among experimental runs, and plant differences (ragweed or sunflower). Thus, the first objective of the study was to determine which of the following drivers determine microbial community composition. These drivers include the: 1) the source community, which was the initial microbial community present in the local soil of each state; 2) the influence of the training plant species (ragweed or sunflower) in the initial phase of the experiment; and 3) the influence of the plant species (ragweed and sunflower) in the final phase of the experiment.

My second objective was to identify the soil bacterial and fungal taxa that were correlated with plant-microbe interactions thought to be indicative of feedback processes. These microorganisms can interact with the plant species in two distinctive phases. First, certain microbial taxa should be selected by either sunflower or ragweed, and these microbial taxa can be identified by their presence and abundance in the pots that only contained ragweed or sunflower. Second, certain microbial taxa should strongly influenced plant growth and plant performance through the mechanism of soil feedback, and these taxa can be identified by their correlations with plant growth. The most interesting taxa in terms of plant-soil feedback are those that strongly associated with their host plants and were also significantly related to the feedback plant received from soil community.

My third objective was to determine whether the key microbial taxa that were significantly related to feedback in 1st experimental is consistently related to feedback in 2nd experimental run, and then to measure the similarity between the dynamics of the key microbial taxa and those of the whole microbial community.

My final objective was to test the hypothesis that bacterial and fungal community variability is correlated with the magnitude of soil feedback scores. I hypothesized that the plants that received strong feedback exerted strong selection pressure on soil communities. Thus, the community that associated with the plant received strong feedback and would be less variable than the community that associated with the plant that received weak feedback.

Material and Methods

2.1 Plant-soil feedback greenhouse experiments

Samples were taken from greenhouse experiments conducted by members of NC1026 at seven locations (Illinois, Montana, Kansas, South Dakota, Oregon, and Michigan). The details of their studies are summarized below.

Weed seed preparation: Seeds of sunflower (*Helianthus annuus*) were collected from Manhattan, Kansas, and seeds of ragweed (*Ambrosia trifida*) were collected from Urbana, Illinois. In preparation for the experiment, sunflower seeds were soaked in 2% of bleach for 20 min in order to break seeds dormancy and protect seeds from fungal attack during soaking. Then seeds were rinsed with distilled water three times for 10 min. Ragweed seeds were vernalized for 3 months at < 4 ℃ in moist sand prior to start of experiment.

Soil preparation: The study soils were collected from local agricultural fields of each location. Soils were mixed 50:50 with sand to facilitate drainage. Michigan collaborators collected two soil types from their experimental research farms: one soil (labeled "agronomy") was from a field in East Lansing, Michigan, with rotation history of soybean – fallow – soybean. The other soil (labeled "Bean and Beet") was from a farm near St. Charles, Michigan with a four-year rotation history of corn, soybean/dry bean, wheat, and sugar beet.

Experiment workflow: The soil feedback experiment had three stages: beginning with 40 pots, the same plant species was grown twice in succession (stages 1 and 2) in order to create different soil microbial community histories by “preconditioning” the soil through exposure to different plants. The pots were then divided among ‘home’ and ‘away’ treatments (stage 3). In ‘home’ treatments ragweed and sunflower were grown in pots

with their own respective histories, i.e. ragweed planted in soils with ragweed history, and sunflower planted in soils with sunflower history (10 replicates). These are called the "ragweed-ragweed" and "sunflower-sunflower" treatments in Figure 6.2. In 'away' treatments, plants were grown in pots with histories of the other plant species (10 replicates), and these are called "ragweed-sunflower" and "sunflower-ragweed" treatments in Figure 6.2. Each plant was allowed to grow for 10 weeks, after which the entire plant was harvested. Illinois, Kansas and Michigan collaborators performed this entire feedback experiment twice, and these different experiments are referred to as "experimental run1" and "run2." Additionally, the Michigan experiment added two control groups besides the regular "home and away" treatments mentioned above: sunflower or ragweed were planted only in stage 3 (i.e. without any soil preconditioning by plants in stages 1 and 2). Thus, Michigan sample sets have six treatments for each experimental run. In summary, the overall experimental design is a 2x2 factorial experiment. One factor is the plant in conditioning phase: ragweed and sunflower; the other factor is plant in feedback phase: ragweed and sunflower. This factorial experiment consisted of four experimental treatments: ragweed-ragweed, ragweed-sunflower, sunflower-sunflower sunflower-ragweed. Each treatment had ten replicates.

Soil collection and plant biomass: From each experiment unit, approximately 27 cm^3 soil was collected after the growth assay, and these soils were stored at -20 ℃ for subsequent analysis. At harvest, plant weight was determined after drying at 60 ℃ for 48 hours. Only plants collected from the third stage (feedback stage) were used to determine feedback score.

Feedback score calculation: In order to attach a feedback score for the soil community in each pot, the baseline biomass of sunflower and ragweed was calculated for each state using the mean biomass of the plants from the respective "away" soils

(sunflower-ragweed and ragweed-sunflower). The feedback score for each plant (i.e. each final pot) was calculated as the biomass of each plant in the "home" treatment minus the baseline biomass for the respective plant species and state, eg. feedback of ragweed-ragweed first pot = biomass of ragweed-ragweed (home soil) first pot - mean biomass of ten sunflower-ragweed (away soil) pots (Figure 6.1).

2.2 Soil microbial community analysis:

In my study, I used soils harvested from above 'home' and 'away' experiment. Soil DNA was extracted from frozen soil using the FastDNA Spin kit for Soil (MP Biomedicals, Solon, OH) according to the manufacturer's instructions. In order to further purify the DNA from soil-associated PCR inhibiting substances, a CTAB (Cetyltrimethylammonium Bromide) cleanup was used: CTAB and NaCl was added to each DNA sample to a final concentration of 1% CTAB and 0.7M NaCl. Samples were incubated at 65 ℃ for 15 minutes, followed by extraction with 24:1 chloroform:isoamyl alcohol and precipitation with 100% EtOH. Pellets were washed twice with 70% EtOH and dissolved in 1X TE buffer to a final DNA concentration of 10 ng/μλ.

Bacterial and fungal community composition was determined using automated ribosomal intergenic spacer analysis (ARISA) (Fisher & Triplett, 1999). This ARISA fingerprint method allows us to analyze soil bacterial communities among different experiment treatments and soil source. The bacterial 16S-23S rRNA intergenic spacer region was amplified with the universal primers 1406F (5'-TGYACACACCGCCCGT-3') and 23SR (5'-GGGTTBCCCCATTCRG-3') targeting the 16S-ITS-23S regions of bacterial *rrn* operons (Fisher & Triplett, 1999); the fungal 18S-ITS1-5.8S-ITS2-28S intergenic spacer regions were amplified using the primers 2234C (5'-GTTTCCGTAGGTGAACCTGC-3') and 3126T (5'-ATATGCTTAAGTTCAGCGGGT-3') (Ranjard et al., 2001). The 5' ends of the

primers 1406f and 3126T were labeled with the fluorochromes 6-FAM (bacteria) and HEX (fungi) to visualize PCR products during capillary gel electrophoresis. Each 50 ul polymerase chain reaction contained 1x Tris buffer, 0.25 mM BSA, 0.25 mM deoxynuclosidetriphosphates, 0.4 μM of each primer, 2.5 mM $MgCl_2$ and 1.25 U Promega GoTaq. The PCR cycling conditions included initial denaturation at 94^{o}C for 2 min, followed by 26 cycles of 94^{o}C for 35 s, 55^{o}C for 45 s, and 72^{o}C for 2 min, with a final extension carried out at 72^{o}C for 2 min. The PCR cycling was performed in an Eppendorf MasterCycler Gradient (Eppendorf AG, Hamburg, Germany). Each DNA samples had three PCR replicates to improve identification of ARISA peaks in profiles.

All DNA fragments generated from ARISA were analyzed by denaturing capillary electrophoresis using an ABI 3730XL Genetic Analyzer (PE Biosystems). Electrophoresis conditions were 63 ℃ and 15 kV with a run time of 120 min using POP-7 polymer. The ROX 1000 size standard (MM-1000-ROX, BioVentures, Inc., Murfreesboro, TN, USA) was used as the internal size standard for the ARISA. ARISA profiles were analyzed using GeneMarker version 1.95 (SoftGenetics, LLC, State College, PA, USA). The size of ARISA fragments ranged from 400 to 1000 base pairs and each fragment of a given size (area in ARISA profile) was taken to represent a different bacterial Operational Taxonomic Unit (OTUs) present in the sample. Peaks with heights higher than 300 relative fluorescence units that occurred in at least two replicates were included in the data analyses (Fisher & Triplett, 1999; Yannarell & Triplett, 2005).

2.3 Statistical analysis:

Multivariate and univariate statistical approaches were used to address the four objectives posed above. Soil microbial community data and factors that comprise the main experimental design were analyzed with the multivariate analysis software,

PRIMER 6 (PRIMER-E Ltd, Plymouth, UK). Statistical analyses were also performed in the R statistical environment using functions in packages *vegan*, *pls* and *MASS* (R Development Core Team, 2009).

***Methods of each objective*:**

Objective one: Which of following drivers is most influential in determining microbial community composition? 1) the source community, which was the initial microbial community present in the local soil of each state; 2) the influence of the training plant species (ragweed or sunflower) in the initial phase of the experiment; and 3) the influence of the plant species (ragweed and sunflower) in the final phase of the experiment.

Soil microbial community composition:

Bacterial and fungal data were analyzed separately. The ARISA area in each sample was measured under the assumption that each area of peak corresponds to a unique taxon (Fisher & Triplett, 1999). In order to reduce run-to-run variability, raw ARISA data from three PCR replicates for each sample were utilized by taking the average height for each peak across all three replicates. Next, ARISA data were transformed by the Hellinger transformation:

$$\sqrt{\frac{height\ of\ peak\ i}{sum(peak\ heights\ in\ this\ sample)}}$$

The Hellinger transformation has been used for two reasons: 1) converting raw peak intensity into relative intensity of peaks controls run-to-run variability in signal strength that may be caused during capillary electrophoresis; 2) as Legendre and Gallagher (Legendre & Gallagher, 2001) recommended for analysis of community composition data, the Hellinger transformation makes long-gradient community composition data (i.e.

many zeros) more amenable to Euclidean-based ordination methods, such as Redundancy analysis (RDA).

After Hellinger transformation of ARISA data, a similarity matrix was calculated using Bray-Curtis coefficient. Non-metric Multi-Dimensional Scaling (NMDS) was used to visualize the patterns in microbial communities associated with sunflower and ragweed among six states. The NMDS method is an ordination technique used to construct a "map" of community relationships in a specified number of dimensions. It creates a map where the distances between each pair of samples on the plot indicate the similarity of the bacterial communities in those samples. The two samples with the highest similarity in the community composition plot closest together; and most dissimilar samples plot furthest apart (Ramette, 2007; Rees et al., 2004).

For community data, the factors - source community, soil training and feedback were included as explanatory variables, which drove microbial community composition. Source community implies the influence of starting soil microbial community; soil training implies the influence of plant species in the soil training phase of the feedback experiment, and feedback indicates the influence of plant species grown during the final phase of the experiment. The influence of each of these drivers was analyzed by partitioning the sum of squares of the ARISA data using permutational multivariate ANOVA (PERMANOVA).

Expectations:

1) If source community is the most influential driver, PERMANOVA will attribute more variation in microbial communities to the source community factor than to the other two factors. Also, in the NMDS plot, the soil communities from the same state will plot together, and communities from different states will plot further apart. This would indicate that the most influential factor for these soil communities is the source community, and the impact of weeds on soil microbial communities is weaker than the

difference between soil sites. If this is the case, in order to eliminate the variance from source community, I will split whole ARISA data state by state to address subsequent goals.

2) If soil training is the most influential driver, PERMANOVA will attribute more variation in microbial communities to the soil training. In NMDS plot, microbial communities will be mostly distinguished by their training history of either ragweed or sunflower. This would indicate that it would take some time for sunflower and ragweed to alter soil microbial communities, longer soil training time in history are driving the soil microbial communities more than initial soil profile and last plant species. This kind of data can be used to find soil microbes which are consistently associated with sunflower and ragweed as the training plant species across all states, as per the next objective.

3) If feedback is the most influential driver, PERMANOVA will attribute more variation in microbial communities to the feedback. In NMDS plot, microbial communities from all states will be distinguished by the last plant species they were exposed to, regardless soil source and training. This would indicate that last plant species changed the soil microbial communities very fast and efficiently, no matter the initial soil profile and plant in training. I can use this data to find soil microbes which are consistently associated with sunflower and ragweed as the last plant species across all states, as per the next objective.

Objective two: Identify the soil bacterial and fungal taxa that are correlated with the two potential interactions between plants and their soil microbial community during the feedback process. Montana samples were not considered in this and all following analyses because the treatments were severely unbalanced due to sample loss.

Microbial taxa correlated to plant species and plant-soil feedback

There are two kinds of potential interactions between plants and their soil microbial communities during the feedback process. First, the presence of sunflower/ragweed causes a change in its associated soil community; then the altered soil communities will generate feedback to host plant, resulting in increased or decreased growth of plant.

Microbial taxa correlated to plant species

For the following analysis, ARISA peaks were used as "taxa". For the first interaction (plants change their associated soil community), in order to eliminate variances introduced by source community, ARISA data were analyzed separately for each state. Microbial taxa strongly associated with ragweed and sunflowers were detected by focusing on the "home" soil (ragweed-ragweed and sunflower-sunflower treatments). Redundancy analysis (RDA) (Borcard et al., 1992) seeks artificial axis that puts each of two treatments at either end, reflecting the strongest difference between ragweed-ragweed and sunflower-sunflower. Each particular taxon was projected on to RDA axis 1, and its value on axis 1 indicates how that taxon's relative abundance changes corresponding to the difference between the treatment. Taxa near the end of the axis are important in explaining ragweed/sunflower differences, and taxa near the center are less important. Thus, the microbial taxa having extreme values (quantile 5%) along axis1 value were categorized as either "strong ragweed-associated taxa" or "strong sunflower-associated taxa".

Microbial taxa correlated to plant soil feedback

The second interaction is that the altered microbial communities generated effects on plant growth. Analyzing this interaction allows us to determine which microbial taxa in the plant-altered soil communities were highly influential in explaining the feedback score of sunflower and ragweed across states. In order to explore the complex relationship between microbial communities and host plant species, a strategy for

identifying feedback-correlated microbial taxa was developed. This strategy involved two main phases:

1. To determine how many “key” microbial taxa in the whole communities were sufficient to explain the feedback score of the host plant, I performed a data reduction by partial least square regression. A strings selections criterion was applied to the result of the partial least square regression, and the taxa that met the criterions were used to model the soil feedback score. This process was repeated with increasing relaxed criterions until the combination of microbial taxa predicted feedback well. Specifically, there are more than 500 microbial taxa in ARISA profiles of whole communities, I want to determine the threshold of reduction of microbial taxa as explanatory variables: 1) the influence of microbial taxa on feedback score were measured and the high influential taxa were selected by a tentative threshold; 2) The correlation between selected microbial taxa and feedback was investigated to check whether this size of selected microbial taxa was sufficient to explain feedback score of host plant; 3) The selection threshold was relaxed until the combination of microbial taxa predicted feedback well.
2. To determine whether the same key microbial taxa would be selected with different sample sets. I performance a sensitivity analysis using a "bootstrap" resampling method with the threshold decided in step 1 to select “key” and sensitive microbial taxa. The "bootstrap" process outline above was repeated 1000 times by subsampling 40 samples from all 60 samples.

As previously mentioned, feedback score was calculated by the following formula: biomass of home soil – mean biomass of away soil. Statistical models treated the feedback score of the home soil pot as the dependent variable and all microbial taxa as independent variables.

Selection of strongly correlated microbial taxa from community

The number of microbial taxa represented by ARISA profiles is usually very large (more than five hundred), while the dependent variable, feedback score, is univariate. Partial least squares regression (PLS) is a statistical technique that generalizes and combines features from principal component analysis and multiple regressions. It is designed to model dependent variables (feedback score) as a function of a very large set of independent variables (microbial taxa). The PLS first performs a simultaneous decomposition of microbial data and feedback scores to search for a set of principal components, which are generated to explain as much of the covariance between microbial data and feedback score as possible. This is followed by a regression step where the principal components of the microbial data are used to model the feedback score. The "key" microbial taxa in predicting feedback were picked out from the whole community based on their loadings on the principal components. Forty samples (two-thirds of the total samples) were randomly selected to serve as a training data set, and 20 samples were used as a model validation data set. The optimal number of components to include in the analysis was determined by leave one-out cross-validation with ten randomly selected subsets of data. Three components were selected because this is the number of components after which the cross-validation error (CV) and percentage of explained variance did not show a significant decrease (Figure A1). The specific loading of each microbial taxon on the components was extracted from the fit model by the function *loadings*. A group of microbial taxa with high loading scores on components represented those with the high correlation to feedback score.

Additional questions I addressed were how many key microbial taxa are sufficient to classify and fit feedback to the plant? And, are a few key microbial taxa sufficient to determine the feedback direction, or is the whole microbial community involved?

Evaluation of selected microbial taxa correlation with feedback

The next step was testing the selected key microbial taxa for their ability to predict feedback scores. Is this particular set of microbial taxa sufficient to predict the direction of feedback in each pot? Here, I treated the feedback as a categorical variable: either positive or negative. Linear discriminant Analysis (LDA) was used to describe the differences in microbial communities between the plants receiving positive and negative feedback, allocating observations into the two groups. This method maximizes the ratio of between-class variance (positive vs. negative) to the within-class variance (within positive or negative group) in microbial data thereby guaranteeing maximal separation between positive feedback communities and negative feedback communities. Plotting the feedback score on the first linear discriminants was shown the predicted classification of feedback direction. A misclassification rate was made by comparing predicted feedback score from LDA model with real feedback score. The misclassification rate can evaluate whether this particular microbial taxa combination provided sufficient sensitivity in allocating feedback score into right direction.

Linear regression for two groups was another method I used to test the relationship between microbial taxa and feedback; I used the general regression model in *glm*. Unlike treating feedback score as categorical data in LDA, I used the feedback score as continuous data in general linear model (GLM). In GLM, the significance of the regression coefficient of one independent variable indicates that this variable has significant relationship with the dependent variable. Thus the significance of the coefficient of a particular microbial taxon indicates whether this taxon is significantly correlated with host plant feedback score. To further identify whether all potential microbial taxa were needed in the regression model, a stepwise model selection process (*step* in *MASS* package) was used to determine the "best" subset taxa according to the Akaike information criterion (AIC). A smaller AIC indicates a better fitness model. *Step* used a backward elimination model search procedure, which began with all N microbial

taxa and dropped the one with insignificant P-value. Then model with the remaining N-1 taxa was fitted and the next dropped taxon was insignificant. This process continues until no further taxa can be dropped. The original model before stepwise selection was called the full model, the model after selection was called the reduced model.

I used the model deviation to assess the quality of these various regression models. The model deviation is a measure of the discrepancy between the data and the model estimates. A small deviation indicates a tight fit of the model to the data. Since the bacterial and fungal communities associated with two plant species may differ in model deviation scale, I used the percentage of variance to standardize the model deviation derived from different microbial communities. Percentage of variance can be explained by the full regression model = 1-(deviation of full model/ deviation of null model). Here, the null model is one presuming that there is no relationship between microbial taxa and feedback score, and the deviation of the null model is derived by setting the regression coefficients of all independent variables (taxa) to zero. Similar to the misclassification rate, the percentage of explained variance is another indicator to evaluate the correlation of one microbial taxa combination with feedback score.

Based on the misclassification rate and the model deviation, if these taxa classified/regressed feedback well, this cutoff loading score was kept and went to taxa sensitivity test in the "bootstrap" PLS; if these taxa did not classify/regress feedback satisfactorily, the cutoff loading score was gradually decreased to include more microbial taxa into RDA and GLM analysis until the microbial taxa combination was sufficient to answer our questions (Table A 1-4).

"Bootstrapping" Partial least Square

"Bootstrap" analysis was used to determine whether the partial least square models supplied robust information about the key microbial taxa from whole community in the study. Will the same key microbial taxa be identified as strongly related to feedback

score if a different sample data are used? To conduct "bootstrapped" PLS, I generated 1000 random data sets consisting of 40 ARISA profiles selected with replacement from a total of 60 ARISA profiles, which are all home samples from all states of one plant species. PLS was performed as previously described. I used the upper 5% quantiles (5% of OTUs maintained) as cutoff loadings score for all three components because my comparison of the different cutoff criteria for microbial taxa showed that this threshold gave good explanation for feedback (Table 6.4 and 6.5). I recorded the number of times each particular microbial taxon was selected as key taxon after 1000 runs. The "bootstrap" method wrapped with PLS was programed by R.

Selection of important microbial taxa in feedback model

The microbial taxa occurring more than 500 times in PLS bootstrap were considered as sensitive taxa with high correlation with feedback scores, because the number of taxa with more than 50% of appearance was similar to the number of taxa with best prediction ability in cutoff loading test. The functions of these taxa in generating positive or negative feedback to ragweed or sunflower were inferred from the sign of their coefficients in the linear discriminant analysis and linear regression models.

For these key microbial taxa, I combined the results of previous analyses to infer the potential roles of the key microbial taxa in plant-soil feedback. The important microbial taxa were selected by following conditions:

1) More strongly associated with one plant species than another plant species (redundancy analysis);
2) More than 50% of appearance in the result of PLS bootstrap;
3) Good predication of feedback score, which were received by the host plant of these microbial taxa across all the states.

Expectations:

See Table 6.1.

Comparison of key microbial taxa with whole soil microbial community

The influence of source community (i.e. state), soil training and feedback to selected key microbial taxa was determined by permutational multivariate ANOVA. A Mantel test was used to measure the correlation between two matrixes: one matrix contained sample distances based on only the selected key microbial taxa that met the ecological conditions described above; the other matrix contained sample distances based on every microbial taxa in the whole soil community. The results of permutational multivariate ANOVA and mantel test allowed me to compare the importance of selected microbial taxa in soil feedback to that of the whole microbial community.

Objective three: Validate the key microbial taxa identified from second object using replicate experiment run.

Illinois, Kansas and Michigan collaborators conducted the feedback experiment twice. In Illinois and Kansas, soil was collected from same location at different times for these two experiments; In Michigan, soil was collected from two locations (agronomy and bean beat farms) at different times for these two experiment runs. Thus, the data of second experiment run were used to validate the significant of microbial taxa identified from second objective. Did the key microbial taxa identified in the first run play a similar role in the second run? Or did the key microbial taxa in plant-soil interactions vary over time?

Linear discriminant analysis (LDA) and general linear regression model (GLM) were used to validate the model. The microbial and feedback data of the second run were applied to LDA model and GLM developed from the data of 1st run. Two indicators were

used to measure the actual consistent capability of the selected microbial taxa. For LDA, a misclassification rate was made by comparing predicted and real feedback score of 2nd run. For GLM, MSPR, which stands for *mean squared predictor error,* was the mean of regression square error of model using 2nd run data. If MSPR are fairly close to MSE (mean square error) based on modeling building data (1st run), then the selected microbial taxa had significant effects on feedback in both runs.

MSPR was calculated by $\text{MSPR} = \frac{\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2}{n}$,

where: Y_i is the value of the feedback in the ith 2nd run sample

$\hat{Y}_i$ is the predicted feedback score for ith 2nd run sample

n is the number of samples in 2nd run data set.

Expectations:

1) Small misclassification rate and MSPR are fairly close MSE based on 1st data model. This would mean that the selected key microbial from the first run were also important in the second run. The source soil microbe communities were different in two runs, but the plant still selectively interacted with the same microbial taxa. The microbe-plant interactions were consistent in time.

2) Large misclassification rate and MSPR is much bigger than MSE based on 1st data model. This would mean that the key microbial taxa from the 1st run were poor predictors for the 2nd experiment run. The different source soil microbe communities generated different microbial taxa with significant effects on host plant feedback. The microbe-plant interactions depended on the source communities and varied in time.

Objective four: Determine whether bacterial and fungal community variability is correlated with the magnitude of soil feedback to plants.

Microbial community variability reflects the control capability of plant to its microbial communities during their interaction process. The altered microbial communities in turn affected host plant presenting as feedback. Thus I hypothesize that the microbial community variability is correlated with the magnitude of feedback. In order to directly compare ragweed and sunflower control capability, I used microbial data from "home" soil where plants experienced the same plant species during soil training. Microbial community variability was measured by Bray-Curtis dissimilarity using *vegdist* and *betadisper* in *vegan* package. The Bray-Curtis dissimilarity was used to calculate the distance between group members and the group centroid on the basis of the multi-dimensional scaling space; thus, the variability of a microbial community was the average distance between each microbial community and community corresponding treatment group centroid. Since every microbial community had ten replicates in soil training-feedback treatment, I used the mean centroid distance of the ten replicates. For example, microbial community variability of ragweed in Illinois= average Bray-Curtis dissimilarity of microbial community in ragweed-ragweed pots in Illinois. The absolute value of feedback score were used as the strength of soil feedback. Next, for testing the relationship between microbial community variability and feedback strength, linear regression was used to model the Bray-Curtis dissimilarity against the absolute feedback score of each state. The significance of the coefficient indicates whether microbial community variability is correlated with magnitude of soil feedback.

Expectation:

1) The microbial community variability was significantly negative correlated with the absolute feedback score. Plants receiving strong feedback produced a "tight" microbial community. This result suggests that plants exert high selection pressure on the microbial community, either accumulating mutualistic or pathogenic microbes, resulting in a less variable microbial community that generates strong feedback to plant growth.

2) There is no significant relationship between microbial community variability and feedback strength. Plants receiving strong feedback did not have less variable microbial communities than those receiving weak feedback. This result suggests that the plant species did not specifically select or accumulate associated microbial taxa in community.

Results

3.1 Which of the following drivers was most influential in determining microbial community composition?

In total, 525 bacterial fragments and 429 fungal fragments were recovered following ARISA analyses from all studied samples. Nonmetric multidimensional scaling ordinations for the Bray-Curtis similarity matrix were created for all ARISA data. The plots show that the source community was the most influential driver for both soil bacterial and fungal communities in six states (Figure 6.3A and Figure 6.3B). The microbial communities from the same source community plot together, while communities from different sources plot apart. In some states, the difference between the communities of two experimental runs was just as big as the difference between states, such as KS and IL (Figure 6.3A and Figure 6.3B). These patterns are supported by variance partitioning analyses, where the largest amounts of variance in bacterial (R^2=0.3769, P<0.001) and fungal (R^2=0.4175, P<0.001) communities were attributed to the source community (Table 6.2- 6.3). Although both the bacteria and fungi are mostly driven by the source community, they do not have the same patterns. For bacterial communities, SD and MI stand apart from the other states along axis 1, while all of the other states assembled together in the middle of axis 1 (Figure 6.3A). Fungal communities from MT, OR, and SD are almost identical, while KS was split into two groups (KS 1st run was like the western states, and the 2nd run was like IL; MI was far from the other states (Figure 6.3B).

Starting the soil community was the predominant explanatory factor in determining microbial community composition, but ragweed and sunflower presence in pots also significantly influenced its soil bacterial and fungal community in permutational multivariate ANOVA (P<0.001). The influence of the plant species in the feedback phase

of the experiment was higher than the influence of the plant species in the soil training phase (Table 6.2- 6.3). Since the impact of plants on soil microbial communities was weaker than the differences of the original starting community, I split the whole ARISA data state by state to address subsequent goals in order to eliminate the variance from the source community.

After eliminating the big variance from the source soil community, significant effects of plant species in the two phases on bacterial and fungal community composition were observed within each state (Tables 6.4- 6.5), although the plant history effect in the second experimental run in Illinois was not statistically significant (P=0.111 for bacteria community and P=0.778 for fungal community). The interactions of the plant in soil training and feedback phase were also significant (P<0.05 for all tests; Tables 6.4-6.5) for both bacterial and fungal communities, although the effect sizes of the interaction were relatively minor. For both bacterial and fungal community compositions, plant species in the feedback phase showed the highest variance among these three factors with the exception of Michigan, where the communities were more influenced during the soil training phase (Tables 6.4-6.5).

3.2 Identification of microbial taxa correlated to plant species and plant-soil feedback

3.2.1 Evaluation of selected microbial taxa correlation with feedback

Two methods, including classification of positive or negative soil community by discriminant analyses (LDA) and fitting the feedback scores with taxa abundance in general linear regression (GLM), were used to test the hypothesis that only a small percentage of microbial taxa were influential in distinguishing the relationship between microbial communities and plant feedback. Both LDA and GLM analyses showed that more microbial taxa could better classify the direction of soil feedback (Figure 6.4 and

6.5).

Overall, the accuracy of predictions of soil feedback to plants was increased by including more microbial taxa in the model (Figure 6.4 and 6.5). Around 50 microbial taxa from each community were found when using a 5% quantile as the cutoff loading score for three components. With this combination of microbial taxa, the RDA classification error rate was down to 0% in both bacterial (dash lines) and fungal (solid lines) feedback to both ragweed and sunflower, except 3% in fungal feedback of sunflower (Figure 6.4 and 6.5). The explained variances for all linear regression models were larger than 80% of the total variance. Thus, classifying feedback as categorical data could distinguish soil feedback to plant species quicker and more accurately than fitting the continual feedback score. Moreover, it seems that 10% of microbial taxa from the complex community (50 selected taxa among more than 500 taxa in total) were sufficient to classify the feedback score of the entire microbial community. Additionally, this indicates that 5% quantile as a cutoff loading was a satisfactory boundary for estimating the number of microbial taxa needed in the LDA and regression models.

Based on the cutoff loading score, selected bacteria and fungi from two plant species had different correlations to the feedback score (Figure 6.4 and 6.5). The lower LDA error rate and higher explainable portion of variance indicated that bacteria and fungi from the ragweed soil communities had better explanatory power to their host plant feedback than those from the sunflower community. Additionally, lower error rates and a higher explainable variance were also observed for fungi (solid lines) compared to bacteria (dash lines), regardless of whether they were from the ragweed or sunflower soil community (Figure 6.4 and 6.5).

3.2.2 Microbial taxa correlated to feedback interactions

A set of key microbial taxa was identified based on the facts that these taxa sensitively responded to the presence of host plants (redundancy analyses), were highly associated with soil feedback, and were robust in resampling ("bootstrap" partial least square regression). Bacterial and fungal communities associated with ragweed and sunflowers were analyzed separately. The bubble plots show the abundance of key microbial taxa in plant “home” soils (Figure 6.6 A, C, E, G). Similarly, those taxa selected by one plant could affect the growth of the other plant species. The key microbial taxa in "away" soil conditioned by the other plant species were also plotted (Figure 6.6 B, D, F, H).

The composition of key microbial taxa associated with ragweed and sunflower varied across the states (Figure 6.6 A-H). Five key bacterial taxa that strongly affected sunflower also had strong effects on ragweed; however, only two key bacterial taxa that strongly affected ragweed also had strong effects on sunflower.

The relationship between the geographic distributions of key fungal taxa and the direction of feedback to plant growth in each state can be observed from the bubble plots of “home” soil (Figure 6.6 A, C, E, G). The fungal communities of sunflower provide the best example. Most of beneficial fungi were found in states where sunflower received positive feedback; while most of deleterious fungi were found in states where sunflower received negative feedback (Figure 6.6 G). The ragweed selected fungi have similar results, except in Illinois. In Illinois, ragweed received positive feedback but most key fungi had negative effects on ragweed. For bacteria, only highly abundant positive taxa skewed to states where host plants received positive feedback; the negative taxa distribution did not follow host plant feedback patterns.

However, in some states there were no consistent relationships between key microbial taxa and feedback, such as ragweed and sunflower in Illinois. Both bacteria and fungi,

regardless whether they were beneficial or deleterious, were almost equally selected in this site.

3.2.3 Comparison of key microbial taxa with the whole soil microbial community

To test whether these key microbial taxa were similar to the whole community in structure and ecology, Mantel tests were performed. A significant relationship (Mantel's correlation coefficient =0.645, P<0.001) was found between bacterial key taxa and the whole bacterial community. The fungal key taxa also had a significant relationship with the whole community (Mantel's correlation coefficient =0.396, P<0.001) (Table 6.8).

The overall variance partitioning results were similar between selected taxa and the whole communities. The source community was still the predominant factor for both selected bacterial and fungal taxa (Table 6.6). However, a difference between bacterial and fungal communities was observed. Three explanatory factors (source community, soil training, and feedback) had a better explanation for key bacterial taxa (45.41%) than for the whole community (36.43%). In contrast, the explanatory factors could explain more variation for the whole community (35.99%) than for the key fungal taxa (28.47%). Regarding the key microbial taxa, the plant in the soil training phase became a more influential factor than in the feedback phase. This is opposite of the results for the whole community (Table 6.4 - 6.5).

3.3 Validation of the selected microbial taxa using a replicate experiment run

I used data from the 2nd experimental runs of IL, KS, and MI to validate the key microbial taxa selected by model based on the 1st run data. Using 2nd run data, the misclassification rates were large (around 50% of the feedback scores were misclassified); and mean squared predictor errors based on the 2nd data run (MSPR) was much bigger than the mean square error (MSE) obtained from the model based on the 1st run data (Table 6.7). Thus the 1st run model failed to classify the soil feedback in the 2nd run.

Different key microbial taxa would be generated based on different sources of microbe communities. The microbe-plant feedback varied across source soils which were collected at different times.

3.4 Correlation of microbial community variability with magnitude of feedback

I hypothesized that there may be a negative relationship between microbial community variability and the magnitude of feedback. Ten replicated microbial communities were assigned as one group in this analysis. The distances between each microbial community and the centroid of their group were calculated. The bacterial community variability was negatively correlated with the degree of feedback (coefficient= -21.889, P=0.0228), but there was no significant relationship between fungal community variability and feedback (Table 6.9). This result suggests that a small variable bacterial community correlated with strong feedback to plant growth.

Discussion

4.1 Bacterial and fungal community compositions

4.1.1 Effects of source community on microbial communities

Both bacterial and fungal communities collected along a gradient of weed distribution showed a clear pattern in which the final community composition in a 30-week experiment was mostly affected by the source soil community. The source soil communities varied with sampling locations (states) and time (experimental runs) (Figure 6.3, Table 6.1 and 6.2). The source community also had the strongest effects on key taxa that sensitively responded to feedback interactions (Table 6.5).

These results agree with another finding that the inherent variation of soil is a powerful driver of the changes of the microbial community in feedback experiments (Harrison & Bardgett, 2010). The large variation of the source community strongly impacts the microbial taxa that are associated with different plant species. Thus, the key microbial taxa of feedback were different in each source community (Figure 6.6). That explains the failure of the model (designed based on the first run of the experiment) validation using the data from the second run of the experiment (Table 6.6). This might be because those effective taxa in one soil are missing or less sensitive to the plant than the other taxa in another source community. For example, the species-specific associations that were formed by the plant and mycorrhizal fungi depend on the initial composition of AMF (Bever, 2002; Bever et al., 1996). Other studies also suggested similar observations of biogeographic variations in soil-microbe effects under different sources of soil (Andonian et al., 2011; Callaway et al., 2011; Reinhart & Callaway, 2006). As an example, *Centaurea solstitialis*-soil feedbacks were different from soils from native and nonnative regions (Andonian et al., 2011).

4.1.2 Effects of plant species on microbial communities

Besides the predominant effects of source communities, ragweed and sunflower also significantly changed bacterial and fungal whole community composition. This is consistent with repeated observations in the feedback studies: compositions of the microbial community were changed as a result of a response to different plant species (Bever, 2003; Bever et al., 1997; Ehrenfeld et al., 2005). Then, the altered microbial communities resulted in feedback to the growth of host and competing plants.

Remarkably, plant species in the feedback phase were more influential than plant species in the training phase, except for the plant species in Michigan (Table 6.3 and 6.4). This observation is similar to the work done by Harrison et al. (Harrison & Bardgett, 2010). They even did not detect bacterial community-level responses to plant species in the soil training phase. After driving a soil community by the plant itself or alien species, the changes of microbial communities caused by plant effects were stronger than in the training phase. This is a common observation, especially when the plant microorganism interaction is an accumulation of certain microbial species, such as species-specific pathogens (Brinkman et al., 2010; Westover & Bever, 2001), or symbiotic fungi colonization (Vogelsang & Bever, 2009). The experiment may need to continue for a long time for the plant to select specific microbes; that is, selection by the plant could continue the previous trends in divergence of microbial communities.

In contrast, soil training accounted for slightly higher variations of key microbial taxa than the plant did in the feedback phase (Table 6.5). The reversal of the plant-dependent driver suggests that the duration of the response by the entire microbial community and the duration of response by the key microbial taxa are different. The whole microbial community mostly responded to the final plant species they associated with. Thus, most microbes would gradually adjust to the plant species that is growing currently. However, the changes of key microbial taxa last longer; therefore, they remained significantly altered by the plant in earlier phases. It was also detected that there are significant

interactions between plant species in the two phases (Table 6.5), indicating that plant species' effects in the final feedback phase could be influenced by soils with different backgrounds.

4.2 Key bacterial and fungal taxa in feedback

Two potential interactions between plants and their soil microbial communities happened during the feedback process. First, the presence of sunflower or ragweed caused changes to the associated soil community; then, the altered soil communities generated feedback to the host plants, resulting in an increased or decreased growth of the plant (Bever et al., 1997; Brinkman et al., 2010). In order to evaluate these two potential interactions between microbes and plant-soil microbe in the taxa level, I tested the response of individual microbial taxa to feedback effects.

4.2.1 Bacterial and fungal taxa correlated with feedback

High abundance and diversity of microorganisms were detected in this study, but surprisingly, 10% of taxa from the whole bacterial and fungal communities were sufficient to classify and fit feedback. In fact, in the ARISA profiles, a large number of microbial taxa were either found only in a small portion of samples, or found in a large portion of samples at very low amounts. These taxa rarely drove the soil feedback and thus were likely to be disregarded in analysis. The taxa identified as key drivers of feedback were often widely distributed and were highly abundant in the communities.

The observation that 10% of taxa were sufficient to classify and fit feedback implies that not all microbial taxa are effective feedback agents. Many microbes did not interact with the plants, or just simply "functioned" in ways that had negligible effects on the overall plant growth and fitness. Some research groups have suggested that there could be considerable complementarity, redundancy (Allison & Martiny, 2008; Konopka, 2009) and dormancy (Lennon & Jones, 2011) in soil microbial communities. In the plant-soil

feedback system, the microbial redundancy might also occur (Van der Putten, Klironomos, et al., 2007). Some microbial species in dormant states may still be detected with DNA-based methods like ARISA. However, these microbial redundancies don't mean that the other 90% of microbial taxa are not necessary in plant-soil feedback systems. These taxa, which did not respond to ragweed or sunflower, may be functional to plant growth if they encounter other plant species. Additionally, more positive effects of plant-AMF symbiosis were found in a very diverse soil, including multiple fungal species and non-mycorrihizal microbes (Hart & Reader, 2002). It was hard to identify the specific functions of the additional non-mycorrihizal microbes (Hart & Reader, 2002). This observation suggests that these additional microbes seem to be redundant but affect plant growth indirectly through supporting plant-AMF symbiosis.

4.2.2 Effects of key bacterial and fungal taxa on feedback

My next step was to understand the characteristics of microbial taxa that sensitively responded to feedback interactions: whether they were associated with the host plant and how they caused the plant-soil feedback effects. In summary, there are two mechanisms that could explain the feedback: the direct effects of microbes to host plant species and the asymmetric fitness effects of microbes on competing plant species. The net feedback depends on the relative strength of effects to host and competing plant species (Bever, 2003; Bever et al., 1997). In order to illustrate the pairwise effects of microbial taxa, I inspected the effects of bacterial and fungal taxa and their abundance in host plant soil as the "direct" feedback, and the effects in competing plant soil as the "indirect" feedback.

Feedback could be caused by beneficial or antagonistic taxa that are predominantly present in the soil of the host plant species. The host plant that associates with more beneficial taxa than antagonistic ones can receive positive feedback. Similarly, a host plant that associates with fewer beneficial taxa than antagonistic ones can receive negative feedback. In the current study, the distribution of key taxa in sunflower Oregon

soil is an example. Positive feedback resulted when the associated beneficial bacteria and fungi dominated over antagonists; meanwhile, these bacteria that were beneficial to sunflower were also found to be harmful to ragweed (taxon 820). Thus, this taxon would serve as a positive taxon to sunflower in Oregon (Figure 6.6 D). It is well documented that feedback is generated by one predominant type of microbe associated with plants (Parker, 2001; Reinhart et al., 2005; Van der Putten, Kowalchuk, et al., 2007). Positive feedback is either related to the relative high density of AMF, (Vogelsang & Bever, 2009) (Klironomos, 2003), or related to symbiotic or free-living nitrogen-fixing microbes (Parker, 2001). In contrast, soil microbes with negative impacts are mainly soil-borne pathogens (bacteria and fungi). Previous studies found that more pathogens were accumulated in the negative feedback soil than that in positive feedback soil (Klironomos, 2002; Westover & Bever, 2001).

A plant, by selecting positive fungi for its competitor, can indirectly facilitate its own competitor's growth. For example, fungal taxa (567 and 560) selected by ragweed showed a positive correlation with sunflower feedback (Figure 6.6 F). In a similar way, a plant can indirectly inhibit a competing plant by selecting taxa that suppress the competing plant, such as the negative effect taxa selected by sunflower in ragweed soil (Figure 6.6 D).

An "asymmetric fitness relationship" means antagonistic organisms can still serve as an agent of positive feedback if they are more harmful to the competing plant species. One example could be the sunflower-soil microbe relationship obtained from South Dakota. Only one bacterium (taxa 823) demonstrated a negative correlation with sunflower while other more negative bacterial taxa associated with ragweed. Sunflower might have a high tolerance to some antagonistic taxa, and therefore these antagonistic taxa could be accumulated without harming the sunflower. These sunflower-selected bacterial taxa had more negative effects on ragweed (taxa 779 and 476). As a result, the net feedback to sunflower was less negative than to ragweed. This asymmetric fitness

mechanism is often observed in invasive plants, which can host high concentrations of generalist pathogenic soil-borne fungi (Inderjit & van der Putten, 2010). However, the accumulated pathogens inhibit native plants more than the invaders, thus leading to negative feedback in native plant species (Mangla et al., 2008; Packer & Clay, 2000). In a similar manner, we could expect negative feedback through changes of the beneficial microbial composition. The invasion of *Amaranthus viridis* in *Acacia* soil was associated with reduced abundance and performance of AMF and rhizosphere microbes. The changes in benefactors contribute to the suppression of invader *Amaranthus viridis* to native *Acacia* (Sanon et al., 2009). However, direct evidence of this kind of interaction was not observed in this study.

4.3 Unexplained feedback based on key taxa

Some feedback can't be clearly explained by the direct effects and asymmetric fitness mechanisms discussed above. There are several possible interpretations for these unexplained feedbacks. Firstly, although the presence of ragweed and sunflower changed the soil microbial community, the impacts of two plant species were small compared to the heterogeneous background of soil microbial communities. Secondly, seed-released nutrients and seed surfaces could select bacteria and fungi during the early stages of my feedback experiment. The selected bacteria and fungi could influence seed degradation rates (Chee-Sanford, 2008; Chee-Sanford et al., 2006), which may lead to different seed initial germinations in pots. For example, some seeds in Montana pots did not germinate, which significantly influenced the plant-soil feedback interactions. Thirdly, plant-specific interactions with the soil microbial community are often expected to happen in the rhizosphere soil (Grayston et al., 1998; Nunan et al., 2005). Interactions of rhizosphere microbes and sunflower (Kamal & Bano, 2008; Staman et al., 2001), and rhizosphere microbes and ragweed (Chee-Sanford et al., 2006) have been observed. It is more difficult to completely detect these fine-scale interactions by analyzing samples from

bulk soil in pots. Therefore, it is very difficult to measure the functional taxa in some soil microbial communities.

4.4 Differences between bacterial and fungal communities

Although the responses of bacterial and fungal communities were highly correlated, there are many differences in their responses. First, based on the same selection criteria, fewer fungal taxa were needed to classify and model plant-soil feedback than bacteria (Figure 6.4- 6.5). The combination of key fungal taxa had a stronger correlation with plant-soil feedback than that of bacterial taxa. Second, more key beneficial fungal taxa were identified from states where plants received positive feedback, while more antagonistic fungal taxa were observed from states where the plant received negative feedback (Figure 6.6). This suggests that direct effects were the major cause of fungal feedback to host plants.

The pattern of bacterial results was not as clear as for fungi (Figure 6.6 E, G). The fungal communities have a lower taxonomic diversity and fewer high abundance taxa than bacterial communities in this study. It seems likely that, in a relative simple community (fungi), the taxa and soil feedback relationship might be clearer. It is possible that the fungi were likely to be shaped by host plant specificity (Halling, 2001) since fungal connection with a plant via the hyphal network represents a more direct and specific association than for bacteria, while the enormous taxonomically diverse bacteria are expected to form more indirect relationships with plant than fungi. Thus, it might be difficult to detect the changes of the bacterial community during the feedback interactions. This result would help explain the observation that the change of bacterial communities was not as significant as changes of fungal communities in other plant-microbial community interaction studies (Bezemer et al., 2006; Lorenzo et al., 2010; Yannarell et al., 2011).

The correlation of key taxa and the whole community was significant based on the Mantel test (Table 6.7). These key taxa are effective indicators of the whole community composition in generating feedback. But the correlation between fungal key taxa and the whole fungal community is weaker than for that of bacteria. This reflects the observation that key fungi were less structured by the three explanatory factors, especial the source community, than that of bacteria (Table 6.5). Factors with a combination of soil texture or chemicals may account for the unexplained variance of key fungi, and future work could focus on teasing apart these factors.

4.5 Correlation of microbial community variability and strength of feedback

The accumulation of specific microbial groups, such as deleterious pathogens (Klironomos, 2002; Mangla et al., 2008) or benefactors (Pringle et al., 2009), is one main mechanism of plant feedback generation. Therefore, the more the similar types (eg. pathogens) of microbial groups that accumulate in a soil community, the stronger the plant feedback generated, and the lower the variability of microbial community composition. Previous work has also indicated that the changes in microbial populations around hosts could lead to homogeneous communities under plant selection pressure (Bever et al., 2010). However, my results did not totally support this hypothesis. Only the bacterial community showed significantly decreased variability with increasing strength of feedback effects, while the fungal community variability was not changed, nor was the feedback strength (Table 6.8). Several reasons might explain the disagreement. There might be a few fungal taxa that are able to generate very strong feedback. Previous studies demonstrated that a single, very efficient AMF species might deliver more benefits to plants than a mixture of AMF species, and that increasing mycorrhizal diversity would not increase benefits to the plant (Edathil et al., 1996). Moreover, AMF alone successfully explained much variance of soil community feedback of plants in previous studies (Klironomos, 2002; Lekberg & Koide, 2005; Zhang et al., 2010).

Furthermore, I found that the feedback to the two weeds in the Michigan experiments was nearly neutral, but the microbial community variability in Michigan was not obviously different from that of the other states. These data might reduce the goodness of fit between feedback and variability.

4.6 Limitations of study

Because of the limitations of ARISA in taxonomic identification (Fisher & Triplett, 1999; Okubo & Sugiyama, 2009), I could not determine if the key taxa identified were plant pathogens or mutualists. More detailed knowledge of the selected key taxa would enrich our understanding of the mechanisms of microbial feedback to plant species. These limitations could be solved by identifying these key species using targeted sequencing methods. In addition, the lack of knowledge about initial soil microbial conditions might also limit my conclusions about microbial species change. Knowing the soil microbial community before plant training might allow me to measure the degree of changes of the microbial community under one plant's influence. This could help me understand the capability of a plant in training associated with the microbial community.

4.7 Implications of study

The results have important implications for investigation and management of agricultural weeds. In this study, I found that the spatial variation of weed feedback is correlated with the source microbial community, the presence and abundance of key microbial taxa, and the differences between bacteria and fungi. Future weed studies might consider the following suggestions.

The local microbial community is an important agent of plant feedback. My results indicate that initial microbial communities significantly influence the microbial taxa that could feed back to alter the growth of weeds. This influence might be even stronger than that of plants (Table 6.3 and 6.4). Thus, the success of weed growth depends on which

microbial communities are available and what interactions these microbes will form with plants. Establishing weed-microbe interactions based on the original soil communities before plant is introduced could help provide an explanation of why one weed can grow better in one area than in another (Andonian et al., 2011). It is also highly recommended to have a specific weed management technique applied to each weed growing area.

There are different mechanisms to generate feedback by key microbial species. For example, the emergence of weeds could be a result of direct effects of weed beneficial microbial taxa or could be caused by indirect effects. The indirect effects are derived from the fact that some weeds have a higher tolerance to soil pathogens than competing plant species, so the weed gains positive feedback. In the areas where feedback is caused by direct effects, identifying the beneficial microbial strains of the weed may be helpful to explain weed performance. In contrast, in sites where indirect effects are dominant, management strategies are complicated. Increasing crop pathogen resistance and removing pathogens might need to be considered simultaneously.

Both bacterial and fungal communities are powerful agents correlated with feedback. Current research has mostly focused on fungal effects on modifying weed populations in agro-ecosystems (Jordan & Huerd, 2008; Jordan et al., 2000; Vatovec et al., 2005); while the bacteria-plant relationships are generally overlooked (Chee-Sanford et al., 2006; Kremer & Kennedy, 1996). However, this study showed that there are significant correlations between bacteria and feedback. Therefore, more studies are needed to understand the critical roles of key bacteria taxa.

Reference

Adjoud, D., Plenchette, C., HalliHargas, R., & Lapeyrie, F. (1996). Response of 11 eucalyptus species to inoculation with three arbuscular mycorrhizal fungi. *Mycorrhiza, 6*(**2**), 129-135.

Agrawal, A. A., Kotanen, P. M., Mitchell, C. E., Power, A. G., Godsoe, W., & Klironomos, J. (2005). Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. *Ecology, 86*(**11**), 2979-2989.

Allison, S. D., & Martiny, J. B. H. (2008). Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 11512-11519.

An, Z. Q., Guo, B. Z., & Hendrix, J. W. (1993). Mycorrhizal pathogen of tobacco - croping history and current crop effects on the mycorrhizal fungal community. *Crop Protection, 12*(**7**), 527-531.

Anderson, R. L. (2007). Crop sequence and no-till reduce seedling emergence of common sunflower (Helianthus annuus) in following years. *Weed Technology, 21*(**2**), 355-358.

Anderson, R. L., Tanaka, D. L., Black, A. L., & Schweizer, E. E. (1998). Weed community and species response to crop rotation, tillage, and nitrogen fertility. *Weed Technology, 12*(**3**), 531-536.

Andonian, K., Hierro, J. L., Khetsuriani, L., Becerra, P., Janoyan, G., Villarreal, D., Cavieres, L., Fox, L. R., & Callaway, R. M. (2011). Range-Expanding Populations of a Globally Introduced Weed Experience Negative Plant-Soil Feedbacks. *Plos One, 6*(**5**), 8.

Ashton, I. W., Miller, A. E., Bowman, W. D., & Suding, K. N. (2008). Nitrogen preferences and plant-soil feedbacks as influenced by neighbors in the alpine tundra. *Oecologia, 156*(**3**), 625-636.

Azania, A., Azania, C. A. M., Alves, P., Palaniraj, R., Kadian, H. S., Sati, S. C., Rawat, L. S., Dahiya, D. S., & Narwal, S. S. (2003). Allelopathic plants. 7. Sunflower (Helianthus annuus L.). *Allelopathy Journal, 11*(**1**), 1-20.

Baysinger, J. A., & Sims, B. D. (1992). Giant ragweed (Ambrosia -trifiad) control in soybean (clycine -max). *Weed Technology, 6*(**1**), 13-18.

Berestetskiy, A. O. (2004). Problems and advances in biological control of weeds with plant pathogenic fungi. *Mikologiya I Fitopatologiya, 38*(**5**), 1-14.

Bever, J. D. (2002). Host-specificity of AM fungal population growth rates can generate feedback on plant growth. *Plant and Soil, 244*(**1-2**), 281-290.

Bever, J. D. (2003). Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytologist, 157*(**3**), 465-473.

Bever, J. D., Dickie, I. A., Facelli, E., Facelli, J. M., Klironomos, J., Moora, M., Rillig, M. C., Stock, W. D., Tibbett, M., & Zobel, M. (2010). Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology & Evolution, 25*(**8**), 468-478.

Bever, J. D., Morton, J. B., Antonovics, J., & Schultz, P. A. (1996). Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. *Journal of Ecology, 84*(**1**), 71-82.

Bever, J. D., Westover, K. M., & Antonovics, J. (1997). Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology, 85*(**5**), 561-573.

Bezemer, T. M., Lawson, C. S., Hedlund, K., Edwards, A. R., Brook, A. J., Igual, J. M., Mortimer, S. R., & Van der Putten, W. H. (2006). Plant species and functional group effects on abiotic and microbial soil properties and plant-soil feedback responses in two grasslands. *Journal of Ecology, 94*(**5**), 893-904.

Blaney, C. S., & Kotanen, P. M. (2002). Persistence in the seed bank: The effects of fungi and invertebrates on seeds of native and exotic plants. *Ecoscience, 9*(**4**), 509-517.

Borcard, D., Legendre, P., & Drapeau, P. (1992). Partialling out the spatial component of ecological variation. *Ecology, 73*(**3**), 1045-1055.

Brinkman, E. P., Van der Putten, W. H., Bakker, E. J., & Verhoeven, K. J. F. (2010). Plant-soil feedback: experimental approaches, statistical analyses and ecological interpretations. *Journal of Ecology, 98*(**5**), 1063-1073.

Buhler, D. (2005). NC1026: Characterize Weed Population Dynamics for Improved Long-Term Weed Management Decision Making (NC202), from http://nimss.umd.edu/homepages/home.cfm?trackID=6699

Buhler., D., Hartzler, R. G., & Forcella, F. (1997). Implications of weed seedbank dynamics to weed management. *Weed Science, 45*(**3**), 329-336.

Burton, M. G., Mortensen, D. A., & Marx, D. B. (2005). Environmental characteristics affecting Helianthus annuus distribution in a maize production system. *Agriculture Ecosystems & Environment, 111*(**1-4**), 30-40.

Caesar, A. (2005). Melding ecology, classical weed biocontrol, and plant microbial ecology can inform improved practices in controlling invasive plant species. *Biological Control, 35*(**3**), 240-246.

Callaway, R. M., Bedmar, E. J., Reinhart, K. O., Silvan, C. G., & Klironomos, J. (2011). Effects of soil biota from different ranges on Robinia invasion: acquiring mutualists and escaping pathogens. *Ecology, 92*(**5**), 1027-1035.

Cardina, J., Johnson, G. A., & Sparrow, D. H. (1997). The nature and consequence of weed spatial distribution. *Weed Science, 45*(**3**), 364-373.

Casper, B. B., Bentivenga, S. P., Ji, B. M., Doherty, J. H., Edenborn, H. M., & Gustafson, D. J. (2008). Plant-soil feedback: Testing the generality with the same grasses in serpentine and prairie soils. *Ecology, 89*(**8**), 2154-2164.

Chee-Sanford, J. C. (2008). Weed seeds as nutritional resources for soil Ascomycota and characterization of specific associations between plant and fungal species. *Biology and Fertility of Soils, 44*(**5**), 763-771.

Chee-Sanford, J. C., Williams, M. M., Davis, A. S., & Sims, G. K. (2006). Do microorganisms influence seed-bank dynamics? *Weed Science, 54*(**3**), 575-587.

Ciarka, D., Gawronska, H., Szawlowska, U., & Gawronski, S. W. (2009). Allelopathic potential of sunflower. I. Effects of genotypes, organs and biomass partitioning. *Allelopathy Journal, 23*(**1**), 95-109.

Collins, H. P., Rasmussen, P. E., & Douglas, C. L. (1992). Crop-rotation and residue management effects on soil carbon and microbial dynamics. *Soil Science Society of America Journal, 56*(**3**), 783-788.

Costacurta, A., & Vanderleyden, J. (1995). Synthesis of phytohormones by plant-associated bacteria. *Critical Reviews in Microbiology, 21*(**1**), 1-18.

Cui, Q. G., & He, W. M. (2009). Soil biota, but not soil nutrients, facilitate the invasion of Bidens pilosa relative to a native species Saussurea deltoidea. *Weed Research, 49*(**2**), 201-206.

Cummings, C. L., Alexander, H. M., Snow, A. A., Rieseberg, L. H., Kim, M. J., & Culley, T. M. (2002). Fecundity selection in a sunflower crop-wild study: Can ecological data predict crop allele changes? *Ecological Applications, 12*(**6**), 1661-1671.

Dalling, J. W., Davis, A. S., Schutte, B. J., & Arnold, A. E. (2011). Seed survival in soil: interacting effects of predation, dormancy and the soil microbial community. *Journal of Ecology, 99*(**1**), 89-95.

Davis, A. S. (2007). Nitrogen fertilizer and crop residue effects on seed mortality and germination of eight annual weed species. *Weed Science, 55*(**2**), 123-128.

Dieleman, J. A., Mortensen, D. A., Buhler, D. D., Cambardella, C. A., & Moorman, T. B. (2000). Identifying associations among site properties and weed species abundance. I. Multivariate analysis. *Weed Science, 48*(**5**), 567-575.

Dieleman, J. A., Mortensen, D. A., Buhler, D. D., & Ferguson, R. B. (2000). Identifying associations among site properties and weed species abundance. II. Hypothesis generation. *Weed Science, 48*(**5**), 576-587.

Diez, J. M., Dickie, I., Edwards, G., Hulme, P. E., Sullivan, J. J., & Duncan, R. P. (2010). Negative soil feedbacks accumulate over time for non-native plant species. *Ecology Letters, 13*(**7**), 803-809.

Edathil, T. T., Manian, S., & Udaiyan, K. (1996). Interaction of multiple VAM fungal species on root colonization, plant growth and nutrient status of tomato seedlings (Lycopersicon esculentum Mill). *Agriculture Ecosystems & Environment, 59*(**1-2**), 63-68.

Ehrenfeld, J. G., Ravit, B., & Elgersma, K. (2005). Feedback in the plant-soil system. *Annual Review of Environment and Resources, 30*, 75-115.

Fierer, N., & Jackson, R. B. (2006). The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America, 103*(**3**), 626-631.

Fisher, M. M., & Triplett, E. W. (1999). Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. *Applied and Environmental Microbiology, 65*(**10**), 4630-4636.

Forcella, F., Wilson, R. G., Dekker, J., Kremer, R. J., Cardina, J., Anderson, R. L., Alm, D., Renner, K. A., Harvey, R. G., Clay, S., & Buhler, D. D. (1997). Weed seed bank emergence across the corn belt. *Weed Science, 45*(**1**), 67-76.

Freckleton, R. P., & Watkinson, A. R. (2002). Are weed population dynamics chaotic? *Journal of Applied Ecology, 39*(**5**), 699-707.

Fu, S. L., & Cheng, W. X. (2004). Defoliation affects rhizosphere respiration and rhizosphere priming effect on decomposition of soil organic matter under a sunflower species: Helianthus annuus. *Plant and Soil, 263*(**1-2**), 345-352.

Fumanal, B., Plenchette, C., Chauvel, B., & Bretagnolle, F. (2006). Which role can arbuscular mycorrhizal fungi play in the facilitation of Ambrosia artemisiifolia L. invasion in France? *Mycorrhiza, 17*(**1**), 25-35.

Furnkranz, M., Muller, H., & Berg, G. (2009). Characterization of plant growth promoting bacteria from crops in Bolivia. *Journal of Plant Diseases and Protection, 116*(**4**), 149-155.

Garcia-Orenes, F., Guerrero, C., Roldan, A., Mataix-Solera, J., Cerda, A., Campoy, M., Zornoza, R., Barcenas, G., & Caravaca, F. (2010). Soil microbial biomass and activity under different agricultural management systems in a semiarid Mediterranean agroecosystem. *Soil & Tillage Research, 109*(**2**), 110-115.

Garibay, S. V., Richner, W., Stamp, P., Nakamoto, T., Yamagishi, J., Abivardi, C., & Edwards, P. J. (2001). Extent and implications of weed spatial variability in arable crop fields. *Plant Production Science, 4*(**4**), 259-269.

Geier, P. W., Maddux, L. D., Moshier, L. J., & Stahlman, P. W. (1996). Common sunflower (Helianthus annuus) interference in soybean (Glycine max). *Weed Technology, 10*(**2**), 317-321.

Gibson, K. D., Johnson, W. G., & Hillger, D. E. (2006). Farmer perceptions of weed problems in corn and soybean rotation systems. *Weed Technology, 20*(**3**), 751-755.

Grayston, S. J., Griffith, G. S., Mawdsley, J. L., Campbell, C. D., & Bardgett, R. D. (2001). Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. *Soil Biology & Biochemistry, 33*(**4-5**), 533-551.

Grayston, S. J., Wang, S. Q., Campbell, C. D., & Edwards, A. C. (1998). Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biology & Biochemistry, 30*(**3**), 369-378.

Halling, R. E. (2001). Ectomycorrhizae: Co-evolution, significance, and biogeography. *Annals of the Missouri Botanical Garden, 88*(**1**), 5-13.

Halloin, J. M., Lee, L. S., & Cotty, P. J. (1991). Pre-ripening damage to cottonseed by aspergillus-flavus is not influenced by seed coat permeability. *Journal of the American Oil Chemists Society, 68*(**7**), 522-523.

Harrier, L. A., & Watson, C. A. (2003). The role of arbuscular mycorrhizal fungi in sustainable cropping systems *Advances in Agronomy, 79* (**79**), 185-225.

Harrison, K. A., & Bardgett, R. D. (2010). Influence of plant species and soil conditions on plant-soil feedback in mixed grassland communities. *Journal of Ecology, 98*(**2**), 384-395.

Harrison, S. K., Regnier, E. E., Schmoll, J. T., & Harrison, J. M. (2007). Seed size and burial effects on giant ragweed (Ambrosia trifida) emergence and seed demise. *Weed Science, 55*(**1**), 16-22.

Harrison, S. K., Regnier, E. E., Schmoll, J. T., & Webb, J. E. (2001). Competition and fecundity of giant ragweed in corn. *Weed Science, 49*(**2**), 224-229.

Hart, M. M., & Reader, R. J. (2002). Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytologist, 153*(**2**), 335-344.

Inderjit, & van der Putten, W. H. (2010). Impacts of soil microbial communities on exotic plant invasions. *Trends in Ecology & Evolution, 25*(**9**), 512-519.

Jordan, N., & Huerd, S. (2008). Effects of soil fungi on weed communities in a corn-soybean rotation. *Renewable Agriculture and Food Systems, 23*(**2**), 108-117.

Jordan, N., Zhang, J., & Huerd, S. (2000). Arbuscular-mycorrhizal fungi: potential roles in weed management. *Weed Research, 40*(**5**), 397-410.

Kamal, J., & Bano, A. (2008). Potential allelopathic effects of sunflower (Helianthus annuus L.) on microorganisms. *African Journal of Biotechnology, 7*(**22**), 4208-4211.

Klironomos, J. N. (2002). Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature, 417*(**6884**), 67-70.

Klironomos, J. N. (2003). Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology, 84*(**9**), 2292-2301.

Konopka, A. (2009). What is microbial community ecology? *Isme Journal, 3*(**11**), 1223-1230.

Kremer, R. J. (1993). Management of weed seed banks with microorganisms. *Ecological Applications, 3*(**1**), 42-52.

Kremer, R. J., Begonia, M. F. T., Stanley, L., & Lanham, E. T. (1990). Characterization of rhizobacteria associated with weed seedlings. *Applied and Environmental Microbiology, 56*(**6**), 1649-1655.

Kremer, R. J., & Kennedy, A. C. (1996). Rhizobacteria as biocontrol agents of weeds. *Weed Technology, 10*(**3**), 601-609.

Legendre, P., & Gallagher, E. D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia, 129*(**2**), 271-280.

Lekberg, Y., & Koide, R. T. (2005). Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. *New Phytologist, 168*(**1**), 189-204.

Lennon, J. T., & Jones, S. E. (2011). Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nature Reviews Microbiology, 9*(**2**), 119-130.

Lorenzo, P., Rodriguez-Echeverria, S., Gonzalez, L., & Freitas, H. (2010). Effect of invasive Acacia dealbata Link on soil microorganisms as determined by PCR-DGGE. *Applied Soil Ecology, 44*(**3**), 245-251.

MacDonald, A. A. M., & Kotanen, P. M. (2010). The effects of disturbance and enemy exclusion on performance of an invasive species, common ragweed, in its native range. *Oecologia, 162*(**4**), 977-986.

Machiavelli, Tejoprakash, N., & Khanna, S. (2008). Influence of diversified cropping pattern on microbial activity and population dynamics in agricultural soils. *Research on Crops, 9*(**3**), 593-598.

MacKay, J., & Kotanen, P. M. (2008). Local escape of an invasive plant, common ragweed (Ambrosia artemisiifolia L.), from above-ground and below-ground enemies in its native area. *Journal of Ecology, 96*(**6**), 1152-1161.

Mangan, S. A., Schnitzer, S. A., Herre, E. A., Mack, K. M. L., Valencia, M. C., Sanchez, E. I., & Bever, J. D. (2010). Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature, 466*(**7307**), 752-U710.

Mangla, S., Inderjit, & Callaway, R. M. (2008). Exotic invasive plant accumulates native soil pathogens which inhibit native plants. *Journal of Ecology, 96*(**1**), 58-67.

McEvoy, P. B. (2002). Insect-plant interactions on a planet of weeds. *Entomologia Experimentalis Et Applicata, 104*(**1**), 165-179.

Mitchell, C. E., Agrawal, A. A., Bever, J. D., Gilbert, G. S., Hufbauer, R. A., Klironomos, J. N., Maron, J. L., Morris, W. F., Parker, I. M., Power, A. G., Seabloom, E. W., Torchin, M. E., & Vazquez, D. P. (2006). Biotic interactions and plant invasions. *Ecology Letters, 9*(**6**), 726-740.

Mitchell, C. E., & Power, A. G. (2003). Release of invasive plants from fungal and viral pathogens. *Nature, 421*(**6923**), 625-627.

Monson, R. K., Lipson, D. L., Burns, S. P., Turnipseed, A. A., Delany, A. C., Williams, M. W., & Schmidt, S. K. (2006). Winter forest soil respiration controlled by climate and microbial community composition. *Nature, 439*(**7077**), 711-714.

Mortensen, K., & Hsiao, A. I. (1987). Fungal infestation of seeds from 7 populations of wild oats (avena-fatua l) with different dormancy and viability characteristics. *Weed Research, 27*(**4**), 297-304.

Myers, M. W., Curran, W. S., Vangessel, M. J., Majek, B. A., Mortensen, D. A., Calvin, D. D., Karsten, H. D., & Roth, G. W. (2005). Effect of soil disturbance on annual weed emergence in the northeastern United States. *Weed Technology, 19*(**2**), 274-282.

Nunan, N., Daniell, T. J., Singh, B. K., Papert, A., McNicol, J. W., & Prosser, J. I. (2005). Links between plant and rhizoplane bacterial communities in grassland soils, characterized using molecular techniques. *Applied and Environmental Microbiology, 71*(**11**), 6784-6792.

Okubo, A., & Sugiyama, S. (2009). Comparison of molecular fingerprinting methods for analysis of soil microbial community structure. *Ecological Research, 24*(**6**), 1399-1405.

Packer, A., & Clay, K. (2000). Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature, 404*(**6775**), 278-281.

Pajevic, S., Borisev, M., Orcic, D., Boza, P., & Nikolic, N. (2010). Photosynthetic and biochemical characteristics of invasive species (Ambrosia artemisiifolia L., Ambrosia trifida L. and Iva xanthifolia Nutt.) depending on soil humidity and phenological phase. *Russian Journal of Ecology, 41*(**6**), 498-505.

Parker, M. A. (2001). Mutualism as a Constraint on Invasion Success for Legumes and Rhizobia. *Diversity and Distributions, 7*(**3**), 125-136.

Pringle, A., Bever, J. D., Gardes, M., Parrent, J. L., Rillig, M. C., & Klironomos, J. N. (2009). Mycorrhizal Symbioses and Plant Invasions. *Annual Review of Ecology Evolution and Systematics, 40*, 699-715.

Quimby, P. C., King, L. R., & Grey, W. E. (2002). Biological control as a means of enhancing the sustainability of crop/land management systems. *Agriculture Ecosystems & Environment, 88*(**2**), 147-152.

R Development Core Team (2011). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Ramette, A. (2007). Multivariate analyses in microbial ecology. *Fems Microbiology Ecology, 62*(**2**), 142-160.

Ranjard, L., Poly, F., Lata, J. C., Mougel, C., Thioulouse, J., & Nazaret, S. (2001). Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: Biological and methodological variability. *Applied and Environmental Microbiology, 67*(**10**), 4479-4487.

Rees, G. N., Baldwin, D. S., Watson, G. O., Perryman, S., & Nielsen, D. L. (2004). Ordination and significance testing of microbial community composition derived from terminal restriction fragment length polymorphisms: application of multivariate statistics. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology, 86*(**4**), 339-347.

Reinhart, K. O., & Callaway, R. M. (2006). Soil biota and invasive plants. *New Phytologist, 170*(**3**), 445-457.

Reinhart, K. O., Packer, A., Van der Putten, W. H., & Clay, K. (2003). Plant-soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. *Ecology Letters, 6*(**12**), 1046-1050.

Reinhart, K. O., Royo, A. A., Van der Putten, W. H., & Clay, K. (2005). Soil feedback and pathogen activity in Prunus serotina throughout its native range. *Journal of Ecology, 93*(**5**), 890-898.

Reynolds, H. L., & Haubensak, K. A. (2009). Soil fertility, heterogeneity, and microbes: towards an integrated understanding of grassland structure and dynamics. *Applied Vegetation Science, 12*(**1**), 33-44.

Reynolds, H. L., Packer, A., Bever, J. D., & Clay, K. (2003). Grassroots ecology: Plant-microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology, 84*(**9**), 2281-2291.

S.J Graystona, C. D. C., R.D Bardgettd, J.L Mawdsleyb, C.D Cleggc, 1, K Ritzc, B.S Griffithsc, J.S Rodwelld, S.J Edwardsd, W.J Daviesd, D.J Elstone, P Millarda. (2004). Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. *Applied Soil Ecology, 25*(**1**), 63-84.

Sanon, A., Beguiristain, T., Cebron, A., Berthelin, J., Ndoye, I., Leyval, C., Sylla, S., & Duponnois, R. (2009). Changes in soil diversity and global activities following invasions of the exotic invasive plant, Amaranthus viridis L., decrease the growth of native sahelian Acacia species. *Fems Microbiology Ecology, 70*(**1**), 118-131.

Schmitt, D. P. (1991). Management of heterodera-glycines by cropping and cultural-practices. *Journal of Nematology, 23*(**3**), 348-352.

Singh, B. K., Bardgett, R. D., Smith, P., & Reay, D. S. (2010). Microorganisms and climate change: terrestrial feedbacks and mitigation options. *Nature Reviews Microbiology, 8*(**11**), 779-790.

Staman, K., Blum, U., Louws, F., & Robertson, D. (2001). Can simultaneous inhibition of seedling growth and stimulation of rhizosphere bacterial populations provide evidence for phytotoxin transfer from plant residues in the bulk soil to the rhizosphere of sensitive species? *Journal of Chemical Ecology, 27*(**4**), 807-829.

Stinson, K. A., Campbell, S. A., Powell, J. R., Wolfe, B. E., Callaway, R. M., Thelen, G. C., Hallett, S. G., Prati, D., & Klironomos, J. N. (2006). Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. *Plos Biology, 4*(**5**), 727-731.

Swanton, C. J., & Weise, S. F. (1991). Integrated weed management - the rationale and approach. *Weed Technology, 5*(**3**), 657-663.

Te Beest, M., Stevens, N., Olff, H., & van der Putten, W. H. (2009). Plant-soil feedback induces shifts in biomass allocation in the invasive plant Chromolaena odorata. *Journal of Ecology, 97*(**6**), 1281-1290.

Tian, D., & Babadoost, M. (2004). Host range of Phytophthora capsici from pumpkin and pathogenicity of isolates. *Plant Disease, 88*(**5**), 485-489.

Torsvik, V., & Ovreas, L. (2002). Microbial diversity and function in soil: from genes to ecosystems. *Current Opinion in Microbiology, 5*(**3**), 240-245.

van der Heijden, M. G. A., Bardgett, R. D., & van Straalen, N. M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters, 11*(**3**), 296-310.

Van der Putten, W. H., Klironomos, J. N., & Wardle, D. A. (2007). Microbial ecology of biological invasions. *Isme Journal, 1*(**1**), 28-37.

Van der Putten, W. H., Kowalchuk, G. A., Brinkman, E. P., Doodeman, G. T. A., van der Kaaij, R. M., Kamp, A. F. D., Menting, F. B. J., & Veenendaal, E. M. (2007). Soil feedback of exotic savanna grass relates to pathogen absence and mycorrhizal selectivity. *Ecology, 88*(**4**), 978-988.

Van der Putten, W. H., & Peters, B. A. M. (1997). How soil-borne pathogens may affect plant competition. *Ecology, 78*(**6**), 1785-1795.

Van der Putten, W. H., Vandijk, C., & Peters, B. A. M. (1993). Plant - specific soil-borne diseases contribute to succession in foredune vegetation. *Nature, 362*(**6415**), 53-56.

Van der Putten, W. H., Vet, L. E. M., Harvey, J. A., & Wackers, F. L. (2001). Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in Ecology & Evolution, 16*(**10**), 547-554.

van Loon, L. C. (2007). Plant responses to plant growth-promoting rhizobacteria. *European Journal of Plant Pathology, 119*(**3**), 243-254.

Vatovec, C., Jordan, N., & Huerd, S. (2005). Responsiveness of certain agronomic weed species to arbuscular mycorrhizal fungi. *Renewable Agriculture and Food Systems, 20*(**3**), 181-189.

Vitousek, P. M., & Walker, L. R. (1989). Biological Invasion by Myrica Faya in Hawai'i: Plant Demography, Nitrogen Fixation, Ecosystem Effects. *Ecological Monographs, 59*(**3**), 247-265.

Vogelsang, K. M., & Bever, J. D. (2009). Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. *Ecology, 90*(**2**), 399-407.

Wang, J. F., Kang, S. Z., Li, F. S., Zhang, F. C., Li, Z. J., & Zhang, J. H. (2008). Effects of alternate partial root-zone irrigation on soil microorganism and maize growth. *Plant and Soil, 302*(**1-2**), 45-52.

Westover, K. M., & Bever, J. D. (2001). Mechanisms of plant species coexistence: Roles of rhizosphere bacteria and root fungal pathogens. *Ecology, 82*(**12**), 3285-3294.

Yannarell, A. C., Busby, R. R., Denight, M. L., Gebhart, D. L., & Taylor, S. J. (2011). Soil bacteria and fungi respond on different spatial scales to invasion by the legume *Lespedeza cuneata*. *Frontiers in Microbiology*, *2*(**127**).

Yannarell, A. C., & Triplett, E. W. (2005). Geographic and environmental sources of variation in lake bacterial community composition. *Applied and Environmental Microbiology*, *71*(**1**), 227-239.

Zhang, Q., Yang, R. Y., Tang, J. J., Yang, H. S., Hu, S. J., & Chen, X. (2010). Positive Feedback between Mycorrhizal Fungi and Plants Influences Plant Invasion Success and Resistance to Invasion. *Plos One*, *5*(**8**).

Figures and Tables

Figures

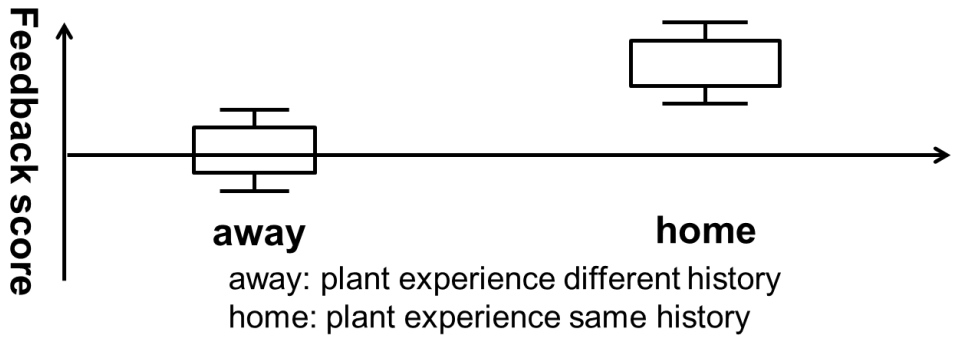


Figure 6.1. Example boxplot of the feedback score of positive feedback plant. Plant-Soil Feedback score = plant biomass in home soil (plants that experienced same plant in soil training) – mean plant biomass in away soil (plants that experienced different plant in soil training)

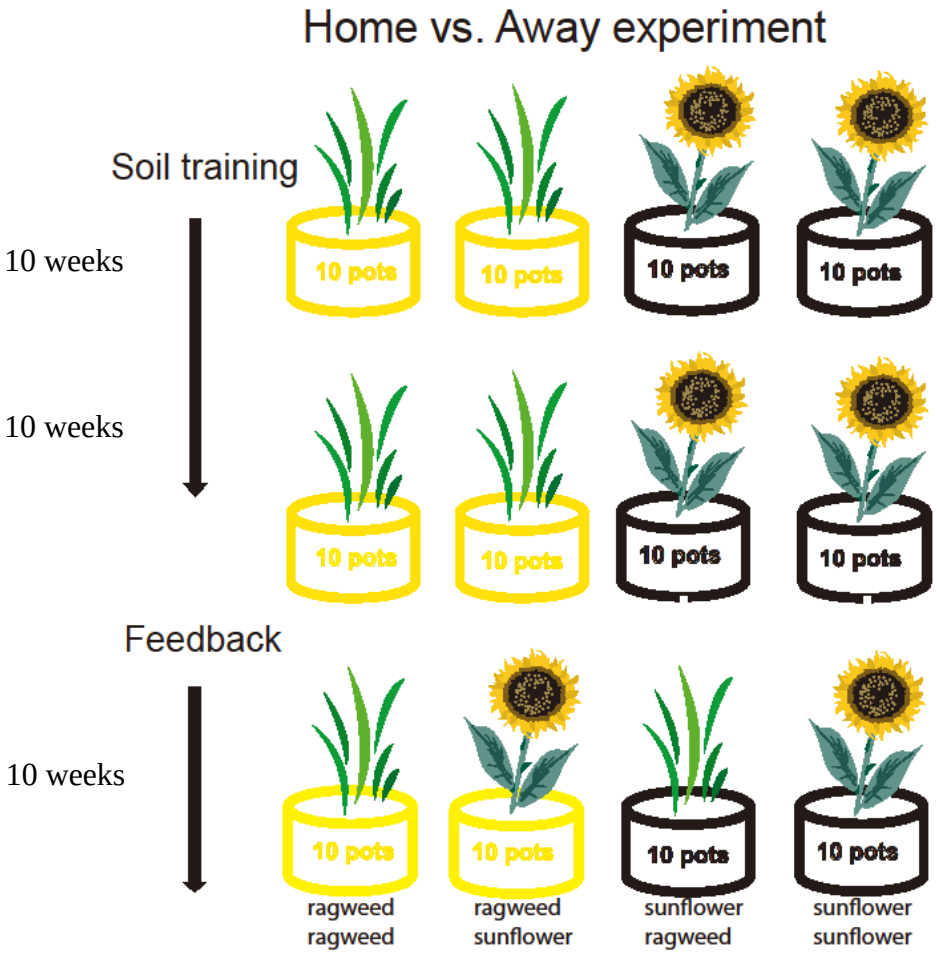


Figure 6.2. Workflow of plant-soil feedback experiment. One pot represents 10 replicates in the experiment. This experiment was conducted in the greenhouses of each state based on the same experimental protocols.

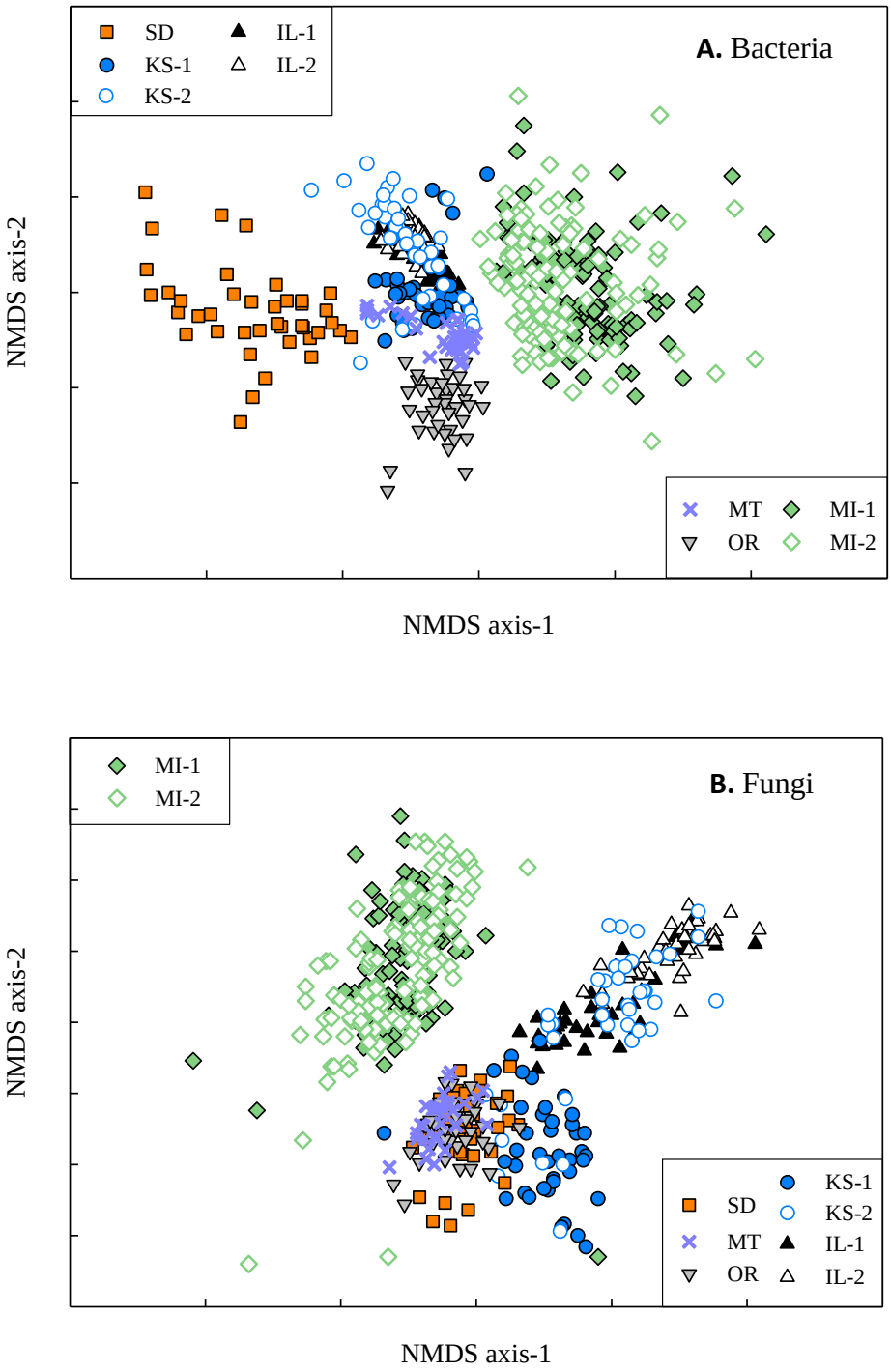
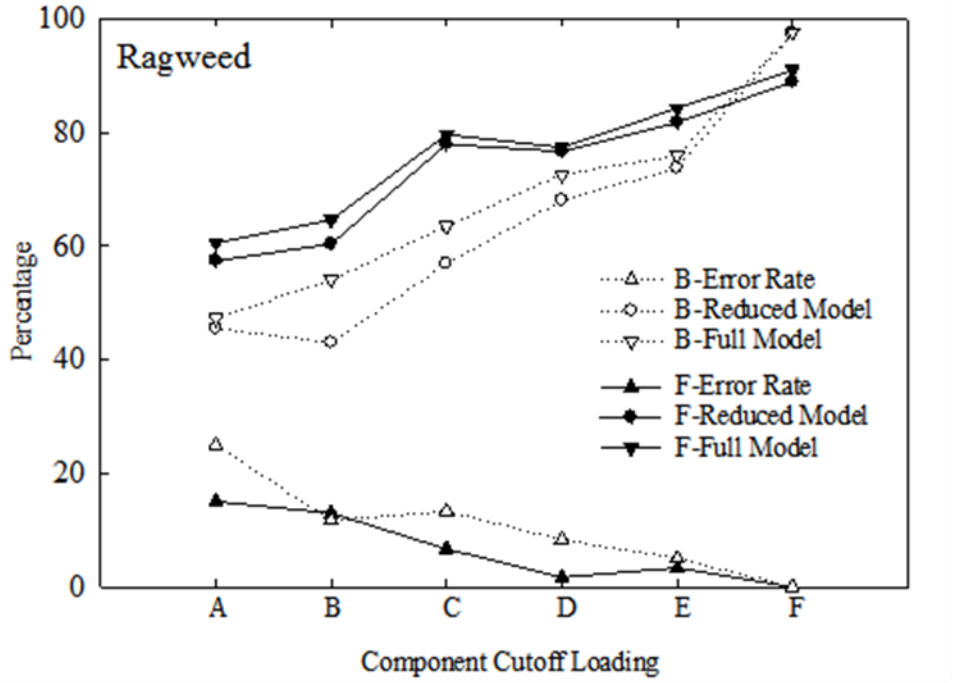
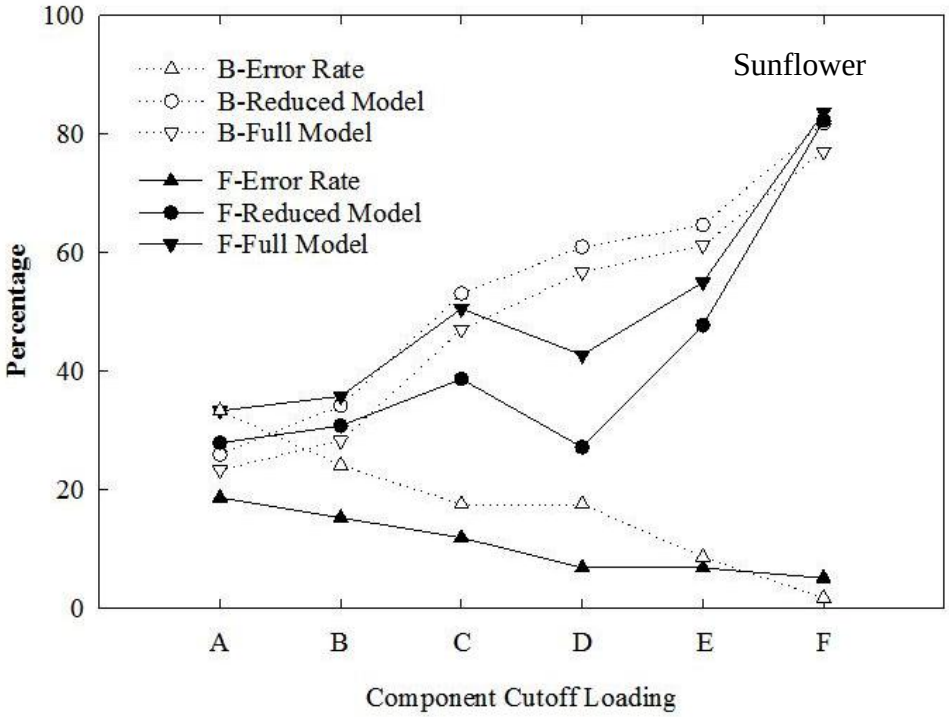


Figure 6.3. NMDS ordination plot of all (A) bacterial and (B) fungal ARISA profiles collected from six states (including replicate experimental runs). Each point represents a microbial community.



	Component cutoff loading					
	A	B	C	D	E	F
	0.2:0.2:0.2	0.175:0.175:0.175	0.15:0.15:0.15	Q5%: 0.2:0.2	Q5%:0.15:0.15	Q5%: Q5%: Q5%
Bacteria #	7	17	25	26	33	55
Fung i#	17	22	31	30	38	46

Figure 6.4. Prediction of linear discriminant analysis and general linear regression to bacterial and fungal taxa associated with ragweed using different cutoff loadings for partial least square components. Upper and lower x-axes indicate the cutoff loading score for three components, number indicates the absolute loading score, Q5% indicates quantile 5% of loading score distribution. Taxa # is the number of microbial taxa included using the above loading score as a limitation. B-: bacteria, F-: fungal. Error rate: linear discriminant analysis error rate. Reduced Model: the variance that can be explained by linear regression on the reduced model after stepwise selection. Full Model: the variance that can be explained by linear regression using all selected taxa at that cutoff.



	Component cutoff loading					
	A	B	C	D	E	F
	0.2:0.2:0.2	0.175:0.175:0.175	0.15:0.15:0.15	Q5%: 0.2:0.2	Q5%:0.15:0.15	Q5%: Q5%: Q5%
Bacteria #	8	12	22	23	32	53
Fung i#	16	17	28	25	33	42

Figure 6.5. Prediction of linear discriminant analysis and general linear regression to bacterial and fungal taxa associated with sunflower using different cutoff loading for partial least square components. Upper and lower x-axes indicate the cutoff loading score for three components, number indicates the absolute loading score, Q5% indicates quantile 5% of loading score distribution. Taxa # is the number of microbial taxa included using the above loading score as a limitation. B-: bacteria, F-: fungal. Error rate: linear discriminant analysis error rate. Reduced Model: the variance can be explained by linear regression reduced model after stepwise selection. Full Model: the variance can be explained by linear regression using all selected taxa at that cutoff.

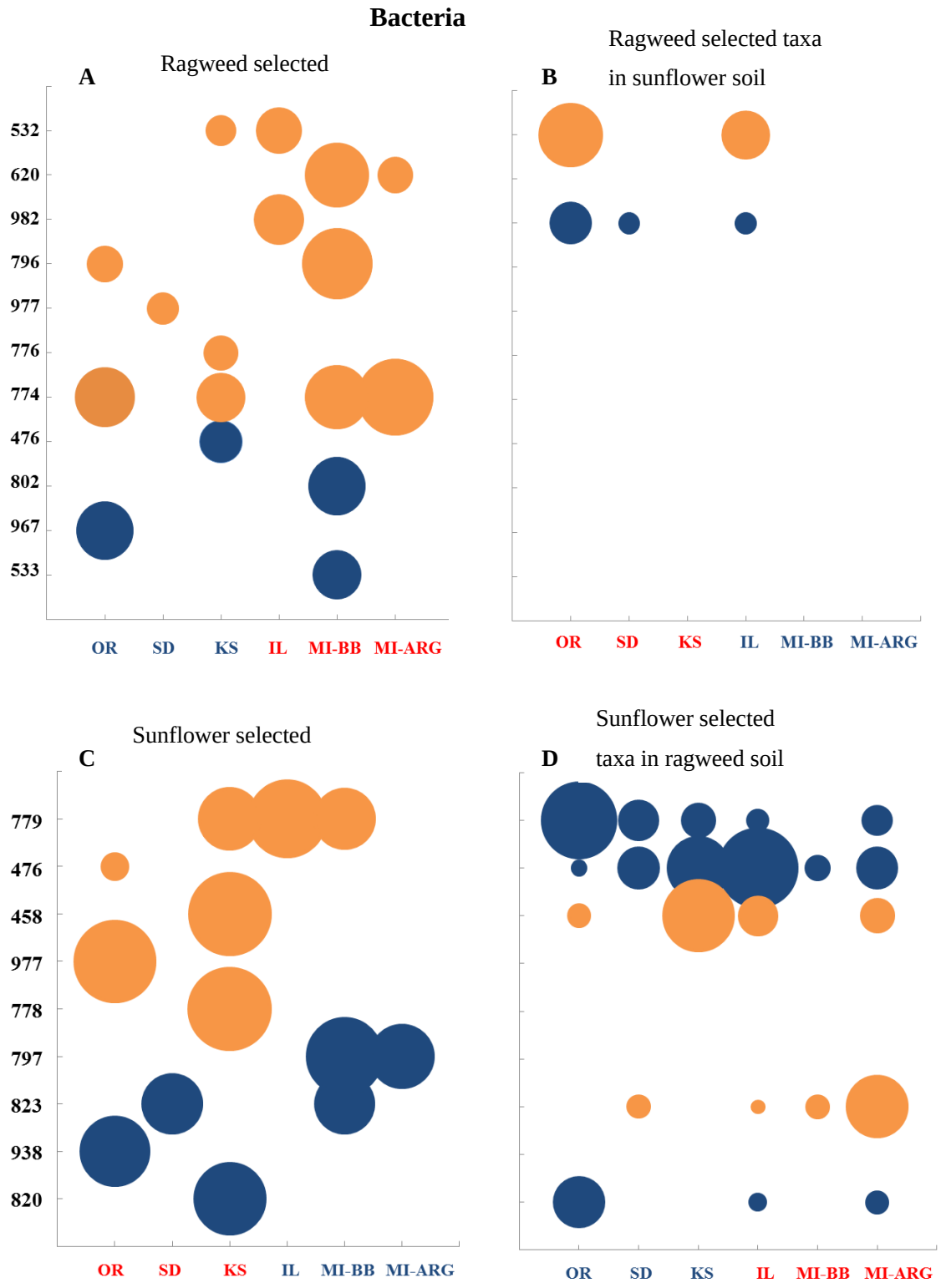


Figure 6.6 (cont.)

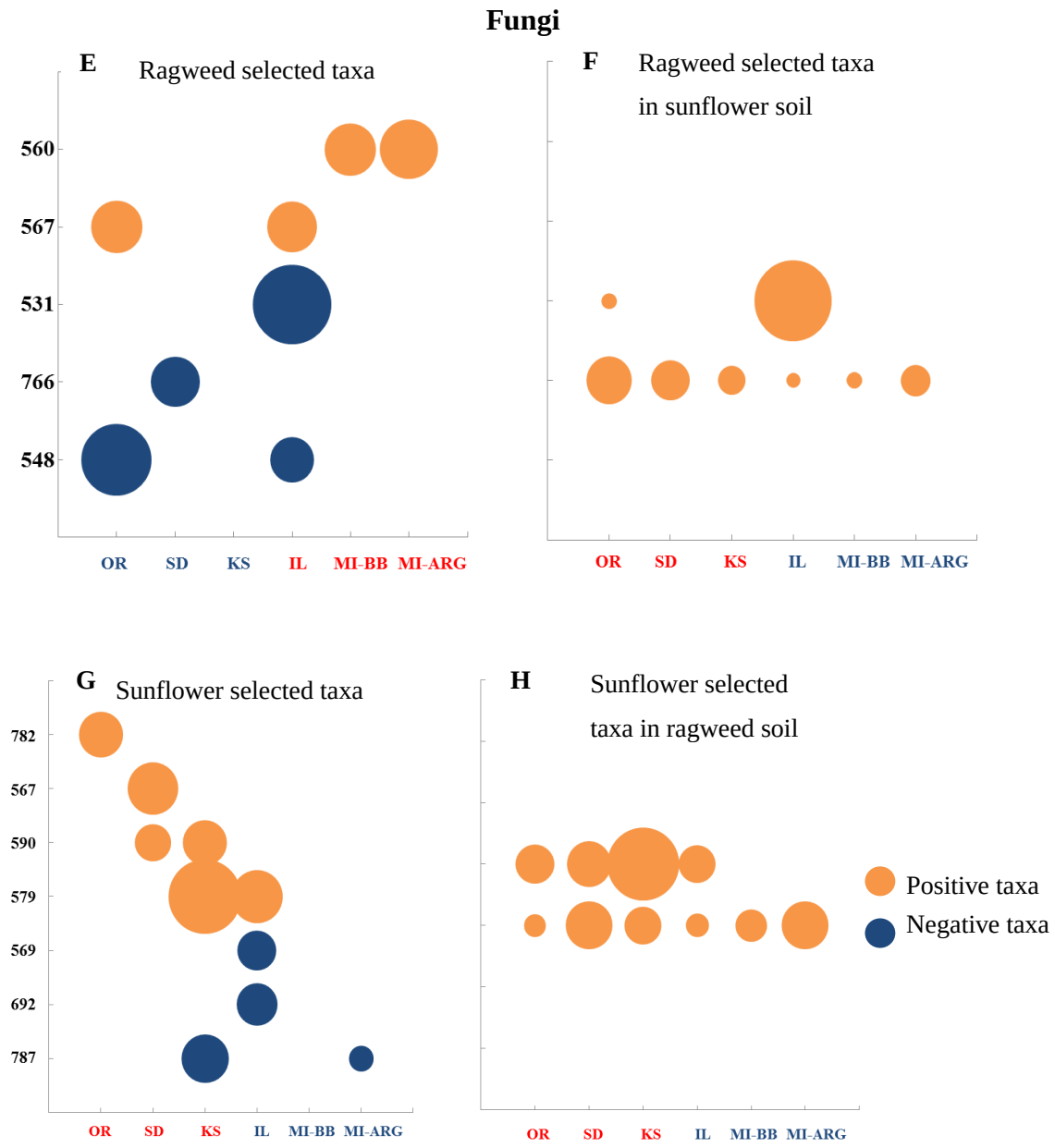


Figure 6.6 Abundance distribution of key bacterial taxa in their host plant soil **(A), (C)** and competing plant soil **(B), (D);** and selected key fungal taxa in their host plant soil **(E), (G)** and competing plant soil **(F) ,(H)** across states. Each bubble represents one microbial taxa, and the size of the bubble is determined by its relative abundance in ARISA profiles. OR: Oregon; SD: South Dakota; KS: Kansas; IL: Illinois; MI-BB: Michigan bean and beat; MI-AGR: Michigan agronomy. Red state abbreviations indicate that the plant received positive feedback in this state, blue state abbreviations indicate that plant received negative feedback in this state. Colored bubbles indicate this microbial taxa was strongly associated with its host plant in the corresponding state. Orange and blue refer to coefficient in regression with feedback, orange: positive; blue: negative.

Tables

Table 6.1. Possible conditions for two competing weeds in the presence of certain microbial taxa. + (positive) and – (negative) indicate the correlation coefficients of the microbial taxon to the feedback score in LDA (linear discriminant analysis) and GLM (general linear regression model). √ indicates the taxon was selected as an important taxon from corresponding treatment soil.

<table>
<tr><td colspan="2">Home soil (self)</td><td colspan="2">Away soil (competitor)</td><td colspan="2">Feedback to plant growth</td></tr>
<tr><td>+</td><td>-</td><td>+</td><td>-</td><td colspan="2"></td></tr>
<tr><td>√</td><td></td><td></td><td>√</td><td colspan="2">Positive feedback to self</td></tr>
<tr><td></td><td>√</td><td>√</td><td></td><td colspan="2">Negative feedback to self</td></tr>
<tr><td rowspan="2">√</td><td rowspan="2"></td><td rowspan="2">√</td><td rowspan="2"></td><td colspan="2">Depend on the relative strength of correlations to self and competitor</td></tr>
<tr><td>Stronger correlation with self</td><td>positive feedback to self</td></tr>
<tr><td></td><td>√</td><td></td><td>√</td><td>Stronger correlation with competitor</td><td>Negative feedback to self</td></tr>
</table>

Table 6.2. The impact of source community (includes different states, fields in MI and experimental run), plant species in soil training, plant species in feedback phases on bacterial community composition. Variation attributed to different variables was partitioned by permutational multivariate ANOVA.

	Df	Sum of square	Mean square	F-value	R^2	P-value
Source community	10	48.69	4.869	25.2804	0.37692	0.001***
Soil training	1	0.552	0.5522	2.8671	0.00427	0.001***
Feedback	1	0.585	0.5854	3.0393	0.00453	0.001***
Residuals	412	79.352	0.1926	0.61427		
Total	424	129.179	1			

Significant code: P-value<0.001 :***, P-value<0.01:**, P-value<0.05:*, P-value<0.1: .

Table 6.3. The impact of the source community (include different states, fields in MI and experimental run), plant species in soil training, plant species in feedback phases on fungal community composition. Variation attributed to different variables was partitioned by permutational multivariate ANOVA.

	Df	Sum of square	Mean square	F-value	R^2	P-value
Source community	10	49.746	4.9746	30.2434	0.41754	0.001***
Soil training	1	0.766	0.7656	4.6545	0.00643	0.001***
Feedback	1	0.532	0.5315	3.2313	0.00446	0.001***
Residuals	414	68.098	0.1645	0.57157		
Total	426	119.141	1			

Significant code: P-value<0.001 :***, P-value<0.01:**, P-value<0.05:*, P-value<0.1: .

Table 6.4. The impact of plant species in soil training, plant species in feedback phases and their interactions within the state on bacterial community composition. Variation attributed to different variables was partitioned by permutational multivariate ANOVA. (Montana samples were excluded because of incomplete sample set).

State	Experimental run	Cropping history	Variables					
			Soil training		Feedback		Soil training X Feedback	
			R^2	P-value	R^2	P-value	R^2	P-value
Illinois	1st	NA	0.049	0.021*	0.122	0.001***	0.059	0.003**
Illinois	2nd	NA	0.036	0.111	0.086	0.001***	0.022	0.484
Kansas	1st	NA	0.057	0.006**	0.090	0.001***	0.042	0.028*
Kansas	2nd	NA	0.109	0.001***	0.066	0.002**	0.0306	0.109
Oregon	1st	NA	0.076	0.001***	0.083	0.001***	0.072	0.001***
South Dakota	1st	NA	0.036	0.046*	0.061	0.001***	0.050	0.005**
Michigan	1st	Agronomy	0.151	0.001***	0.092	0.001***	0.082	0.001***
Michigan	2nd	Agronomy	0.140	0.001***	0.053	0.001***	0.076	0.001***
Michigan	1st	Bean and beet	0.137	0.001***	0.027	0.009**	0.058	0.001***
Michigan	2nd	Bean and beet	0.131	0.001***	0.031	0.001***	0.076	0.001***

Significant code: P-value<0.001 :***, P-value<0.01:**, P-value<0.05:*, P-value<0.1: .

Table 6. 5. The impact of plant species in soil training, plant species in feedback phases and their interactions within state on fungal community composition. Variation attributed to different environmental variables was partitioned by permutational multivariate ANOVA.

(Montana samples were excluded because of incompletes sample set).

State	Experimental Run	Cropping history	Soil training		Feedback		Soil training X Feedback	
			R^2	P-value	R^2	P-value	R^2	P-value
Illinois	1st	NA	0.060	0.002**	0.071	0.003**	0.102	0.001**
Illinois	2nd	NA	0.017	0.778	0.078	0.001***	0.034	0.127
Kansas	1st	NA	0.060	0.002**	0.109	0.001***	0.048	0.004**
Kansas	2nd	NA	0.075	0.001**	0.084	0.001***	0.092	0.001***
Oregon	1st	NA	0.038	0.036*	0.264	0.001***	0.026	0.132
South Dakota	1st	NA	0.057	0.001***	0.184	0.001***	0.069	0.001***
Michigan	1st	Agronomy	0.142	0.001***	0.030	0.001*	0.056	0.002***
Michigan	2nd	Agronomy	0.193	0.001***	0.050	0.001***	0.056	0.001***
Michigan	1st	Bean and beet	0.109	0.001***	0.050	0.012**	0.054	0.001***
Michigan	2nd	Bean and beet	0.127	0.001***	0.017	0.311	0.070	0.001***

Significant code: P-value<0.001 :***, P-value<0.01:**, P-value<0.05:*, P-value<0.1: .

Table 6.6. Summary of impact of source community, soil training and feedback to key microbial taxa and the whole community by permutational multivariate ANOVA.

	Source community		Soil training		Feedback		Partitioned variance by exploratory variables
	R^2	P	R^2	P	R^2	P	
Selected bacterial community	0.4339	0.001	0.0110	0.002	0.0091	0.001	45.41%
Whole bacterial community	0.3489	0.001	0.0073	0.003	0.0080	0.002	36.43%
Selected fungal community	0.2647	0.001	0.0104	0.011	0.0095	0.019	28.47%
Whole fungal community	0.3405	0.001	0.0066	0.002	0.0127	0.001	35.99%

significant code: P-value<0.001 :***, P-value<0.01:**, P-value<0.05:*, P-value<0.1: .

Table 6.7. Validation of the key microbial taxa using replicate experiment run (Illinois 2nd run, Kansas 2nd run, Michigan 2nd run). Misclassification rate is the rate of wrong predictions using 2nd run data based on 1st run model by LDA. MSPR is the mean of regression square error of model using 2nd run data based on 1st run model by GLM. MSE is the mean of regression square error of 1st run model.

	Plant Species-Microbial Community			
	ragweed-bacteria	sunflower-bacteria	ragweed-fungi	sunflower-fungi
Misclassification rate	0.525	0.475	0.475	0.575
MSPR(MSE)	4398.89(54.26)	9042.30(304.36)	1516.51(77.46)	7141.05(535.54)

Table 6.8. Correlation of key taxa and whole communities measured by Mantel test.

Community	Mantel test (correlation coefficient)
Key bacteria Whole bacteria	0.645, P<0.001
Key fungi Whole fungi	0.396, P<0.001

Table 6.9. Relationship of microbial community variability to magnitude of feedback measured by linear regression.

	Coefficient	R2	P
Bacteria	-21.889	0.2561	0.0228*
Fungi	-0.8008	0.000175	0.956

Significant code: P-value<0.001 :***, P-value<0.01:**, P-value<0.05:*, P-value<0.1: .

Appendix A

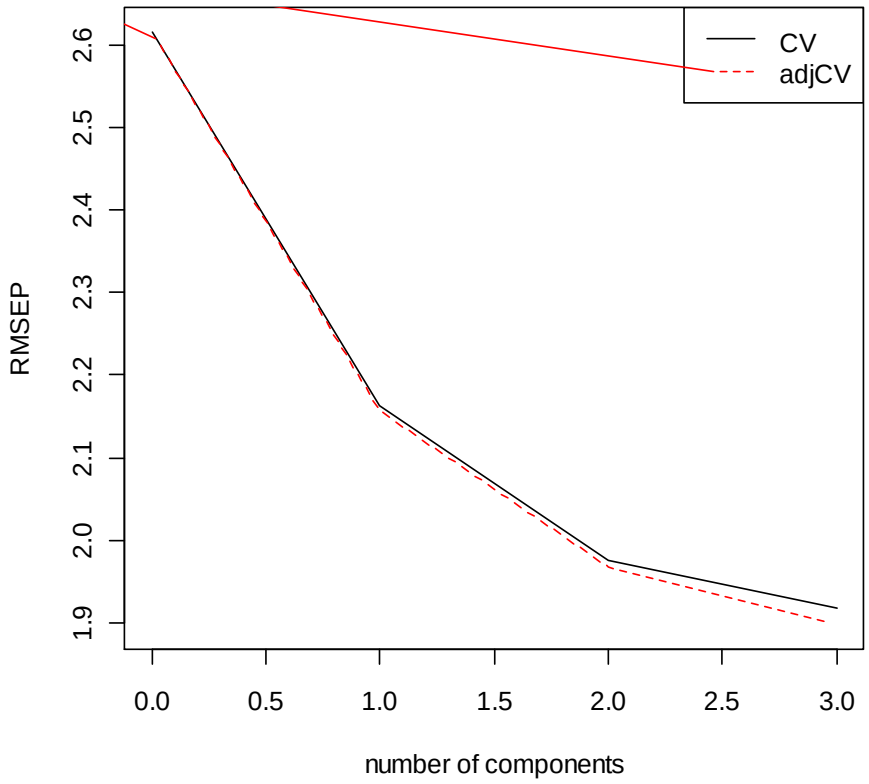


Figure A 1: Example of cross-validation error (CV) plot of partial least square using sunflower bacterial ARISA data. Three components were selected because the cross-validation error (CV) did not show a significant decrease.

Table A 1: The list of bacterial taxa associated with feedback to ragweed was selected by a series cutoff of loading the score of three components in partial least square analysis. Microbial taxa were labeled by their length in ARISA profiles (eg. 555). The ARISA data used "home soil" of five states: plants that experienced same plant in soil training. Fragment was selected if any absolute loading score of the three components was larger than the indicated cutoff score (eg. 0.2). Quantile 5% sampled 5% quantiles corresponding to the distribution of ARISA fragments' loading score of this component. These selected fragments were used to determine how many fragments were sufficient to explain the feedback score.

component 1,	0.2,	0.175,	0.15,	quantile 5%	quantile 5%	quantile 5%
component 2,	0.2,	0.175,	0.15,	0.2,	0.15	quantile 5%
component 3	0.2	0.175	0.15	0.2	0.15	quantile 5%
	555	527	527	532	527	471
	663	532	532	533	532	487
	715	555	533	551	533	508
	760	568	555	555	551	525
	767	607	568	607	555	527
	774	620	607	620	568	532
	780	663	620	663	607	533
		670	663	715	620	536
		715	670	729	663	539
		760	715	747	670	551
		767	760	760	715	555
		774	767	767	729	568
		780	774	774	747	607
		786	780	779	760	617
		790	786	780	767	620
		812	790	786	774	637
		836	792	790	779	663
			796	799	780	670
			812	802	786	710
			833	812	790	715
			836	833	792	721
			851	851	796	729
			864	864	799	747
			918	933	802	757
			982	949	812	760
				982	833	767
					836	774
					851	776
					864	779

					918	780
					933	786
					949	790
					982	792
						796
						799
						802
						803
						805
						812
						833
						834
						836
						844
						851
						859
						864
						902
						918
						933
						949
						952
						967
						977
						981
						982
# taxa were selected		7	17	25	26	33	55

Quantile 5% loading score for three components

	component1	component2	component3
2.50%	-0.13902	-0.09891	-0.10298
97.50%	0.089945	0.108052	0.100618

Table A 1 (cont.)

Table A 2: The list of bacterial taxa associated with feedback to sunflower was selected by a series cutoff of loading score of three components in partial least square analysis.

component 1,	0.2,	0.175,	0.15,	quantile 5%	quantile 5%	quantile 5%
component 2,	0.2,	0.175,	0.15,	0.2,	0.15	quantile 5%
component 3	0.2	0.175	0.15	0.2	0.15	quantile 5%
	555	555	532	730	531	458
	774	572	534	766	532	471
	780	742	555	774	534	531
	797	766	572	779	551	532
	820	774	607	977	555	534
	883	780	706	555	572	540
	933	797	730	788	607	551
	977	820	742	792	706	555
		883	766	812	730	572
		917	774	747	731	579
		933	779	532	742	607
		977	780	820	747	663
			788	823	766	670
			792	841	774	706
			797	851	779	725
			812	883	780	730
			820	895	788	731
			883	534	792	742
			895	551	797	747
			917	780	807	758
			933	797	812	766
			977	977	820	774
				555	823	778
				531	841	779
				607	851	780
				797	883	782
				917	885	788
				706	895	789
				933	917	792
					933	797
					951	807
					977	812
						816
						820
						823
						826
						831
						841

						851 857 883 885 895 900 902 917 933 938 949 951 965 977 997
# taxa were selected	8	12	22	23	32	53

	c1	c2	c3
2.50%	-0.09185	-10.49%	-0.10909
97.50%	0.136849	10.01%	0.101771

Table A 2 (cont.)

Table A 3: The list of fungi taxa associated with feedback to ragweed was selected by a series cutoff of loading score of three components in partial least square analysis.

component 1,	0.2,	0.175,	0.15,	quantile 5%	quantile 5%	quantile 5%
component 2,	0.2,	0.175,	0.15,	0.2,	0.15	quantile 5%
component 3	0.2	0.175	0.15	0.2	0.15	quantile 5%
	447	564	454	435	454	445
	533	676	564	447	564	454
	542	447	572	450	572	547
	554	533	630	455	630	550
	557	542	636	528	636	564
	560	552	676	533	676	572
	567	554	932	542	932	576
	579	556	933	552	933	593
	650	557	447	554	435	620
	766	560	533	556	447	630
	885	567	542	557	450	636
	531	579	552	560	455	648
	548	634	554	567	528	676
	561	650	556	577	533	787
	566	766	557	579	542	932
	569	885	560	616	552	933
	600	531	567	619	554	435
		548	579	634	556	447
		561	634	650	557	450
		566	650	728	560	455
		569	728	766	567	528
		600	766	829	577	533
			829	885	579	542
			885	968	616	552
			968	531	619	554
			531	548	634	556
			548	561	650	557
			561	566	728	560
			566	569	766	567
			569	600	829	577
			600		885	579
					968	616
					531	619
					548	634
					561	650
					566	728
					569	766
					600	829

						885 968 531 548 561 566 569 600
# taxa were selected	17	22	31	30	38	46

	c1	c2	c3
2.50%	-0.13021	-0.11821	-0.13228
97.50%	0.1207	0.127874	0.09659

Table A 3 (cont.)

Table A 4: The list of fungi taxa associated with feedback to sunflower was selected by a series cutoff of loading score of three components in partial least square analysis.

component 1,	0.2,	0.175,	0.15,	quantile 5%	quantile 5%	quantile 5%
component 2,	0.2,	0.175,	0.15,	0.2,	0.15	quantile 5%
component 3	0.2	0.175	0.15	0.2	0.15	quantile 5%
	447	447	447	447	447	447
	531	531	531	450	450	450
	542	542	542	454	454	454
	548	548	548	455	455	455
	557	557	552	531	531	515
	560	560	557	542	542	531
	566	566	560	548	548	532
	567	567	564	557	552	533
	569	569	566	560	557	542
	579	579	567	564	560	548
	600	600	569	566	564	550
	616	616	572	567	566	552
	684	631	579	569	567	557
	700	684	582	572	569	560
	705	700	600	579	572	564
	787	705	616	590	579	566
		787	631	600	582	567
			650	616	590	569
			684	684	600	572
			688	688	616	579
			692	700	631	581
			700	705	650	582
			705	728	684	590
			748	787	688	600
			750	926	692	616
			785		700	631
			787		705	650
			926		728	664
					748	682
					750	684
					785	688
					787	692
					926	700
						705
						728
						748
						750
						769
						785
						787
						899
						926

# taxa were selected	16	17	28	25	33	42

	c1	c2	c3
2.50%	-0.11167	-0.12579	-0.12259
97.50%	0.126979	0.128994	0.118554

Table A 4 (cont.)

Table A 5: The list of bacterial and fungal taxa that appeared more than 500 times in a total of 1000 in partial least square (PLS) "bootstrap" with replacement sampling. The ARISA data used “home soil” of five states: plants that experienced same plant in soil training. Microbial taxa were labeled by their length in ARISA profile. Bacterial taxa to ragweed refers to bacterial taxa selected from the ragweed soil community, others are the same. These microbial taxa with high sensitivity were used to measure the coefficient in discriminant analysis and linear regression model following.

Bacterial taxa to ragweed	Total selected times	Bacterial taxa to sunflower	Total selected times	Fungal taxa to ragweed	Total selected times	Fungal taxa to ragweed
780	1000	780	998	560	1000	447
607	998	555	986	566	1000	557
620	998	779	953	447	999	579
555	997	531	948	579	999	600
786	997	532	947	548	998	700
714	996	933	941	600	998	560
758	995	883	933	557	996	616
663	994	797	930	531	995	705
774	977	788	929	634	965	787
533	972	789	927	554	964	564
532	970	820	915	542	953	531
967	966	977	912	649	951	567
851	952	851	911	552	949	454
802	921	607	908	450	939	572
812	898	458	896	564	928	688
790	897	778	896	606	925	926
767	876	730	858	676	907	566
982	852	725	823	455	873	590
779	821	551	811	533	852	542
507	803	766	801	616	848	548
551	796	747	790	567	799	569
841	796	572	789	561	777	533
528	792	774	778	569	764	728
792	790	841	772	766	753	684
536	769	706	753	932	661	650
729	764	823	748	454	642	532
803	726	790	678	619	638	552
470	718	812	668	572	623	631
527	708	917	663	648	612	746
747	708	476	643	728	578	692
568	699	480	633	631	544	570
796	696	581	623	609	541	455
476	695	637	611	451	537	
799	693	742	594	933	537	
977	690	938	580	597	505	
670	659	527	575			
776	600	469	563			

864	598	534	560
933	585	895	512
793	537	799	501
833	531	663	500

Table A 5 (cont.)